THE UNIVERSITY OF ALBERTA

QUANTITATIVE DETERMINATION OF SOME QUATERNARY AMMONIUM COMPOUNDS

by

(C) KEN OSAMU [illegible], B.Sc.

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

FACULTY OF PHARMACY
AND PHARMACEUTICAL SCIENCES

EDMONTON, ALBERTA

SPRING, [illegible]

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FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Quantitative Determination of Some Quaternary Ammonium Compounds", submitted by Ken Osamu Okamura in partial fulfillment of the requirements for the degree of Master of Science.

ABSTRACT

A dye partition technique was employed to estimate quantitatively some medicinally important quaternary ammonium compounds. Methyl orange, bromthymol blue and Orange IV were used as complexing dyes for the quaternary ammonium agents. The complex formed was measured colourmetrically.

Linear relationships between concentration and absorbance were obtained for the majority of quaternary ammonium substances investigated. Using the calibration curves obtained a quantitative procedure was developed for pharmaceutical dosage forms. Satisfactory results were obtained in the majority of dosage forms investigated. The tablet excipients which were studied had negligible effects on the procedure. The results obtained with this method were compared to the results by an official procedure or the manufacturer's method, where this was possible.

The theoretical aspects of this procedure are discussed.

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. L. G. Chatten for the invaluable assistance and encouragement provided during the course of this project.

The author also acknowledges the financial assistance given him by the Faculty of Pharmacy in the form of assistantships.

The organic medicinals and their dosage forms were generously supplied by the following pharmaceutical companies: Winthrop Laboratories (ambenonium chloride, Mytelase 10 mg. and 25 mg. tablets, penthienate bromide, Monodral 5 mg. tablets and Monodral elixir); Parke, Davis & Company Ltd. (benzethonium chloride, Phemerol solution and tincture); Merck, Sharp & Dohme of Canada Ltd. (bethanecol chloride, demecarium bromide, Humorsol ophthalmic solution 0.25%); The Wm. S. Merrell Co. (cetylpyridinium chloride, Cepacol solution and lozenges); Ciba Company Ltd. (chlorisondamine chloride, domiphen bromide, Bradosol powder and lozenges, oxyphenonium bromide, Antrenyl 5 mg. and 10 mg. tablets); Ayerst Laboratories (echothiophate iodide, Phospholine eyedrops 3.0 mg., 6.25 mg. and 12.5 mg., valethamate bromide); Hoffman-LaRoche Ltd. (edrophonium bromide, Tensilon injection, pyridostigmine bromide, Mestinon 60 mg. tablets); Abbott Laboratories Ltd. (hexocyclium methosulfate,

Tral with phenobarbital tablets); Smith, Kline & French Interamerican Corporation (isopropamide iodide, Darbid 5 mg. tablets); Lakeside Labroratories Ltd. (mepenzolate bromide, Cantil 25 mg. tablets, pipenzolate bromide, Piptal 5 mg. tablets); G.D. Searle & Company of Canada Ltd. (methantheline bromide, Banthine 50 mg. tablets, propantheline bromide, Pro-Banthine 7.5 mg., 15 mg. and 30 mg. tablets, Pro-Banthine injection); Poulenc Limited (pentolinium tartrate, Ansolysen 40 mg. tablets and Ansolysen injection); John Wyeth & Brother (Canada) Ltd. (trimethidinium methosulfate, Ostensin 20 mg. tablets).

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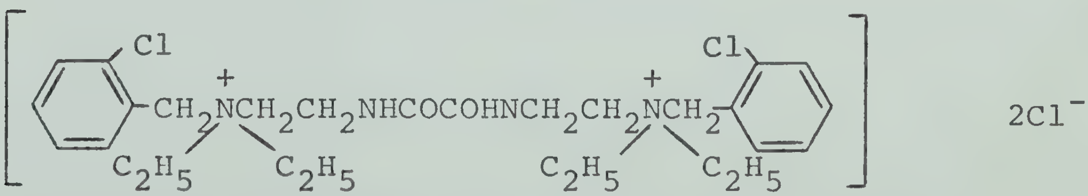
INTRODUCTION AND LITERATURE SURVEY

Interest in the biological activity of quaternary ammonium salts stems from the work of Crum Brown and Fraser (1), who in 1868 were the first to record the curariform activity of certain alkaloids. Quaternary ammonium compounds emerged into greater importance in 1916 when Jacobs _et al_ (2-4) discovered the germicidal activity of the hexamethylenetetramine series. The most important contribution was made simultaneously by Barlow and Ing (5), and by Paton and Zaimis (6) in 1948. They discovered the neuromuscular and ganglionic blocking activity of the polymethylenebistrimethyl ammonium salts. Quaternary ammonium compounds have found clinical value as antispasmodic and antisecretory agents in the treatment of various irritant gastric conditions. More recently quaternary ammonium compounds have also been found to be antifungal.

The compounds presently available which were used in this study are:

1) Ambenonium Chloride (Mytelase)

N,N'-Bis(2-diethylaminoethyl) oxamide bis(2-chlorobenzyl Chloride).

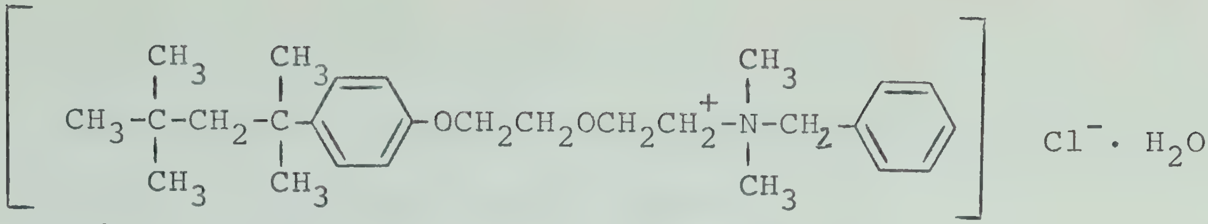


Molecular weight - 608.5

Pharmacological action - cholinesterase inhibitor.

2) Benzethonium Chloride (Phemerol Chloride)

Benzyldimethyl{2-[2-(_p_-1,1,3,3-tetramethylbutylphenoxy) ethoxy]-ethyl}ammonium chloride.

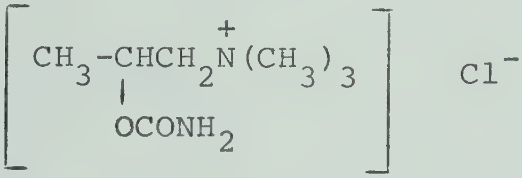


Molecular weight - 466.09

Pharmacological action - topical antiseptic

3) Bethanecol Chloride (Urecholine Chloride)

(2-hydroxypropyl) trimethylammonium chloride carbamate.

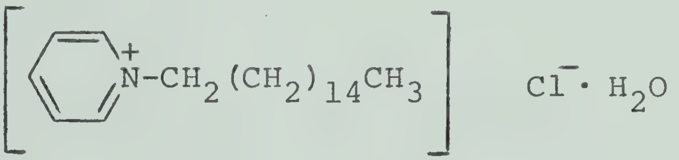


Molecular weight - 196.68

Pharmacological action - cholinergic agent.

4) Cetylpyridinium Chloride (Cepacol Chloride)

1-hexadecylpyridinium chloride.

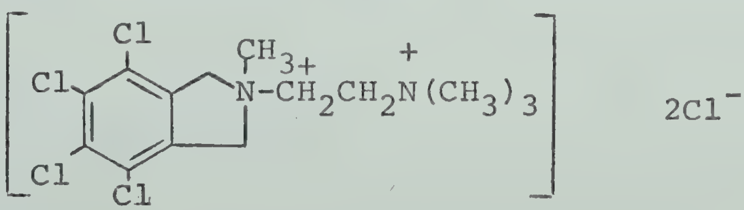


Molecular weight - 357.99

Pharmacological action - antiseptic.

5) Chlorisondamine Chloride (Ecolid Chloride)

4,5,6,7-tetrachloro-2-(2-dimethylaminoethyl)-2-methyl-isoindolinium chloride methochloride.

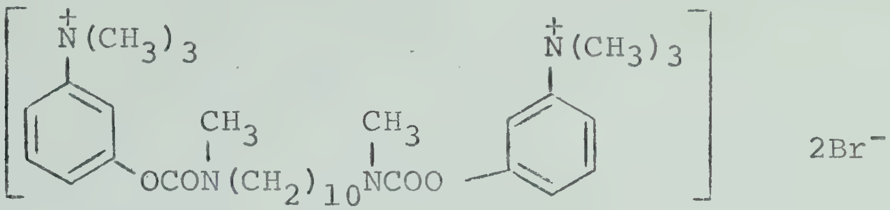


Molecular weight - 429.07

Pharmacological action - ganglionic blocking agent.

6) Demecarium Bromide (Humorsol)

N,N'-bis(3-trimethylammoniumphenoxycarbonyl)-N,N'-dimethyldecamethylenediamine dibromide.

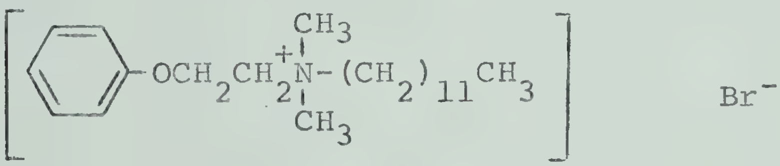


Molecular weight - 716.61

Pharmacological action - parasympathomimetic agent.

7) Domiphen Bromide (Bradosol Bromide)

Dodecyldimethyl (2-phenoxyethyl) ammonium bromide.

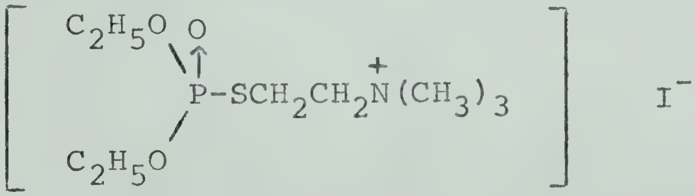


Molecular weight - 414.46

Pharmacological action - antiseptic.

8) Echothiophate Iodide (Phospholine Iodide)

(2-Mercaptoethyl trimethylammonium iodide) 0,0-diethyl phosphorothioate.

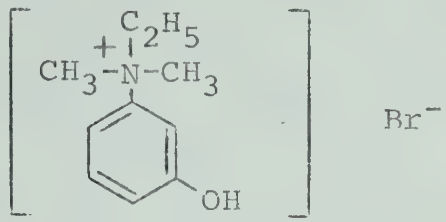


Molecular weight - 383.23

Pharmacological action - a cholinesterase inhibitor in glaucoma therapy.

9) Edrophonium Bromide (Tensilon)

Ethyl (m-hydroxyphenyl) dimethylammonium bromide.

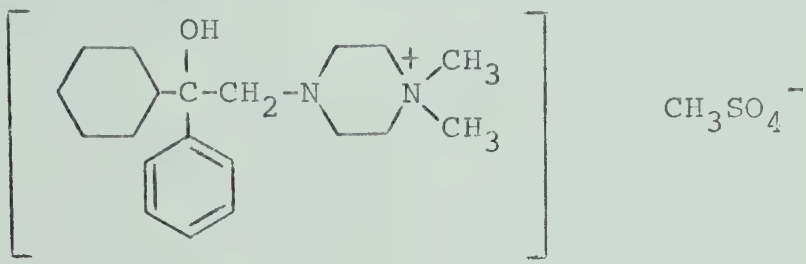


Molecular weight - 246.15

Pharmacological action - parasympathomimetic agent.

10) Hexocyclium Methosulfate (Tral)

N-(β-Cyclohexyl-β-hydroxy-β-phenethyl)-N'-methyl piperazine methosulfate.

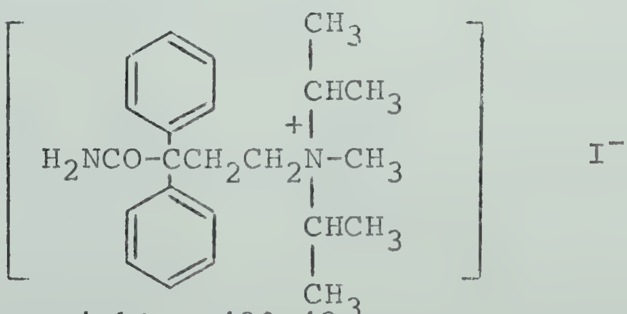


Molecular weight - 428.61

Pharmacological action - parasympatholytic agent.

11) Isopropamide Iodide - (Darbid)

(3-carbamoyl-3,3-diphenylpropyl)diisopropyl methyl ammonium iodide.

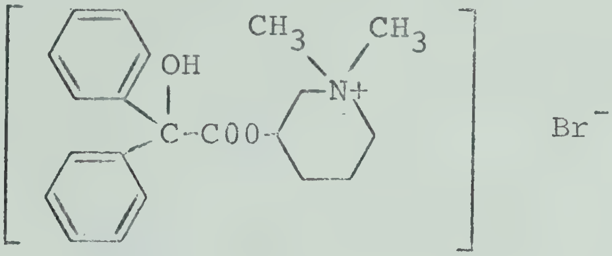


Molecular weight - 480.42

Pharmacological action - parasympatholytic agent.

12) Mepenzolate Bromide (Cantil)

N-methyl-3-piperidyl benzilate methyl bromide.

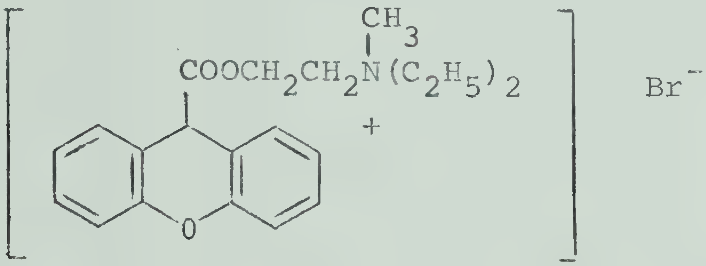


Molecular weight - 420.37

Pharmacological action - anticholinergic agent.

13) Methantheline Bromide (Banthine Bromide)

Diethyl(2-hydroxyethyl)methylammonium bromide xanthene-9-carboxylate.

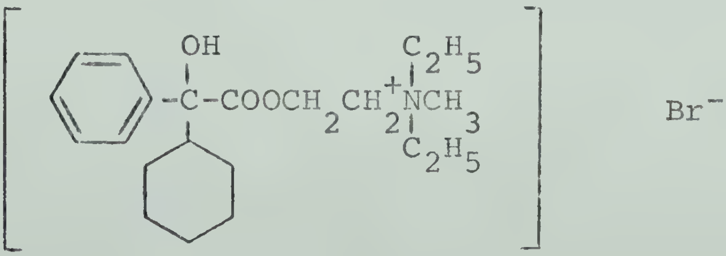


Molecular weight - 420.34

Pharmacological action - parasympatholytic agent.

14) Oxyphenonium Bromide (Antrenyl)

Diethyl(2-hydroxyethyl)methylammonium α-phenylcyclohexane glycolate bromide.



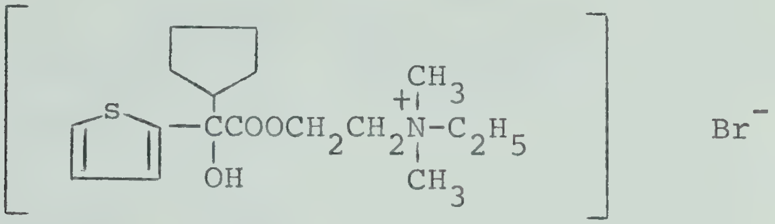
Molecular weight - 428.41

Pharmacological action - anticholinergic agent.

15) Penthienate Bromide (Monodral Bromide)

Diethyl (2-hydroxyethyl) methylammonium bromide,

cyclopentyl-2 thiophene glycolate.

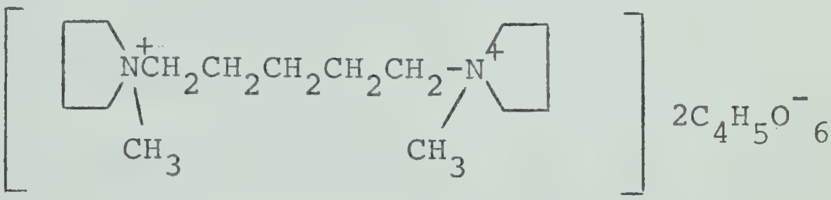


Molecular weight - 420.41

Pharmacological action - anticholinergic agent.

16) Pentolinium Tartrate (Ansolysen Tartrate)

1,1'-Pentamethylene bis (1-methylpyrrolidinium hydrogen tartrate)

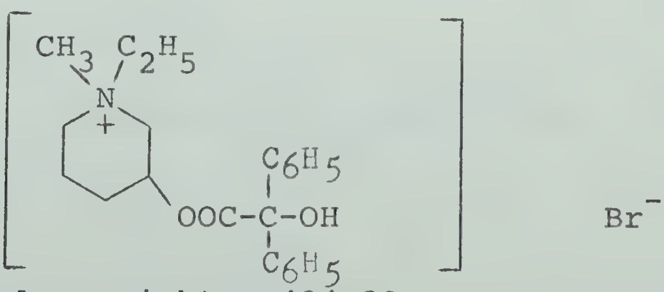


Molecular weight - 538.58

Pharmacological action - ganglionic blocking agent.

17) Pipenzolate Bromide (Piptal)

1-Ethyl-3-hydroxy-1 methylpiperidinium bromide benzilate.

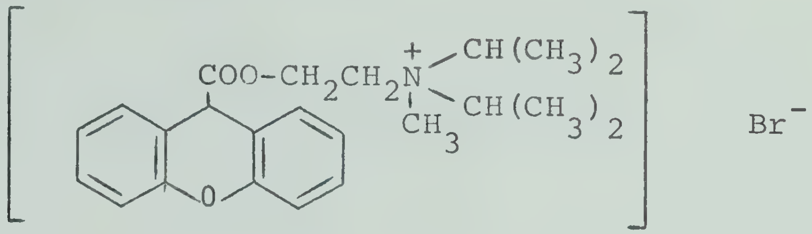


Molecular weight - 434.39

Pharmacological action - anticholinergic agent.

18) Propantheline bromide (Pro-Banthine)

(2-Hydroxyethyl)diisopropyl methylammonium bromide xanthene-9-carboxylate.

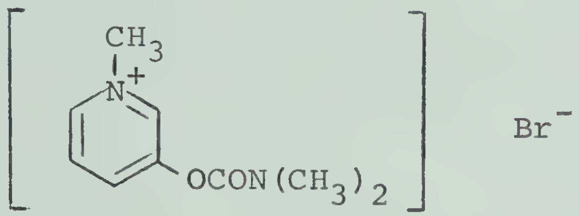


Molecular weight - 448.42

Pharmacological action - anticholinergic agent.

19) Pyridostigmine bromide (Mestinon bromide)

3-Hydroxy-1-methylpyridinium bromide dimethyl carbamate.

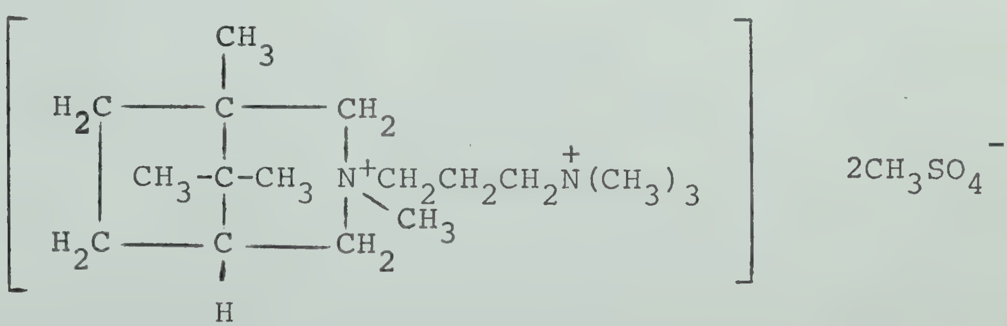


Molecular weight - 261.14

Pharmacological action - parasympathomimetic agent.

20) Trimethidinium methosulfate (Ostensin)

N-(γ-trimethylammonium propyl)-N-methylcamphidinium dimethylsulfate.

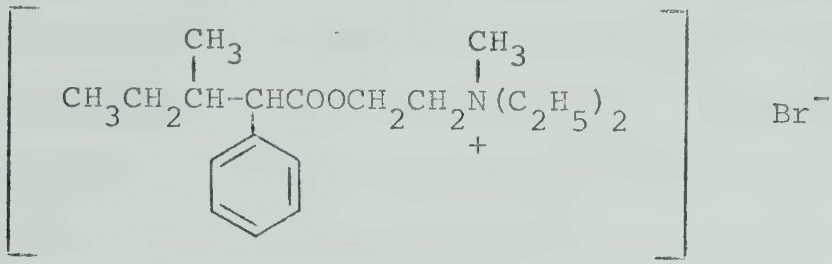


Molecular weight - 490.67

Pharmacological action - ganglionic blocking agent.

21) Valethamate bromide (Murel)

3-methyl-2-phenylvaleric acid diethyl (3-hydroxyethyl) methammonium bromide ester.



Molecular weight - 386.38

Pharmacological action - antispasmodic, anticholinergic.

The quantitative analysis of quaternary ammonium compounds began about 1938 when Prudhomme (7) discovered that alkaloids form chloroform soluble complexes with acid indicators such as eosin. The absorbance of the chloroform solution was found to be quantitative. Auerbach (8), in 1943 described a method in which buffered solutions of quaternary ammonium compounds were complexed with bromphenol blue, and the complex extracted with ethylene dichloride. In 1947, Colichman (9) applied the indicator displacement effect to the direct photometric determination of two surface active agents by using an excess of bromphenol blue, and measuring the colour of the aqueous solution. Ballard, Isaacs and Scott (10) determined several quaternary ammonium compounds which included pentolinium tartrate, decamethonium bromide and hexamethonium bromide. In their method the compound was complexed with bromthymol blue and extracted with chloroform. The chloroform was filtered through glass wool into a solution containing boric acid in ethanol. The extinction of

this solution was measured against a chloroform blank at 420 mμ. They obtained linear calibration curves but their study was not extended to pharmaceutical dosage forms.

Two photometric methods were presented by Helgren, Theivagt and Campbell (11), in 1954 for the analysis of hexocyclium methosulfate. Bromthymol blue was used as the colour producing agent in one instance and ammonium cobaltothiocyanate was used in the other. Reiss (12), presented a method in which the quaternary ammonium compounds were converted to iodides by potassium iodide. The iodide was oxidized by nitrous acid to iodine which was estimated colourimetrically.

Santoro (13), in 1960, selectively determined isopropamide iodide in the presence of amine bases. In this method the substance was complexed with methyl orange in pH 10.2 buffer and the complex extracted into chloroform. Re-extraction of the chloroform solution gave a colour in the aqueous layer which was measured spectrophotometrically.

The most intensive work in this field has been by Schill and his co-workers. They have published a series of papers on the photometric determination of amines and quaternary ammonium compounds with bromthymol blue (14-16). Papers have also been published on the determination of quaternary ammonium compounds with hexanitrodiphenylamine (17) and by their picrates (18). These methods were, however, non-specific because amines would complex under the same conditions.

Deppeler and Becker (19), in 1964, investigated the use of dithizone as a complexing reagent for the analysis of

quaternary ammonium compounds. Chin and Lach (20) presented a method based on the reaction of picric acid with the quaternary ammonium compound in alkaline medium to form a coloured complex which was measured spectrophotometrically after extraction with chloroform. However, their study was limited to only a few quaternary ammonium compounds.

In a more recent investigation, Irving and Markham (21) utilized bromocresol green as a complexing agent in analyzing longchain tertiary alkylamines and quaternary ammonium salts (tri-n-octylamine and tetra-n-hexylammonium iodide). This method suffers from the disadvantage of having a blank which depended both on the pH and on the concentration of the excess reagent.

Ultraviolet spectroscopy has also been used for the analysis of certain quaternary ammonium compounds. Pernarowski and Chatten (22) determined four germicidal compounds and their dosage forms by ultraviolet spectroscopy. Kracmar and Zyka (23) employed this technique in 1961 for the estimation of methantheline and propantheline and their dosage forms. They obtained satisfactory results in all instances except for the duplex coated tablets. In 1968, Varcel (24) devised a mathematical equation which would eliminate interferences from excipients in coated tablets.

Chafetz (25) used ultraviolet spectroscopy for the estimation of penthienate bromide. In this method the substance was oxidized with periodic acid or ceric sulfate which greatly increases their sensitivity in the ultraviolet region.

The B.P. (26) employs ultraviolet spectroscopy for the assay of pyridostigmine tablets, and the U.S.P. XVII (27) utilizes the same principle for edrophonium injection. The N.F. XII (28) describes an ultraviolet procedure for penthienate bromide. Although the ultraviolet method is very sensitive and rapid, interferences result from the excipients in certain dosage forms.

Several volumetric methods have been employed for the analysis of certain quaternary ammonium compounds. Pifer and Wollish (29) in 1952, titrated quaternary ammonium compounds in non-aqueous solvent with perchloric acid in dioxane. The titration could be carried out potentiometrically, or visually using crystal violet indicator. Caswell (30) presented a method in which the halogen anion could be titrated with silver nitrate either electrometrically or visually using dichlorofluorescein indicator. Carkhuff and Boyd (31), in 1954 assayed cetylpyridinium chloride by titrating it with sodium lauryl sulfate, using methyl yellow as the indicator.

An official procedure for benzethonium is described in the N.F. XII (28). This method is a two phase titration which employs sodium tetraphenylboron as titrant and bromphenol blue as the indicator. Chloroform is used as the organic phase. A similar procedure was proposed by Billow and Baker (32) for benzethonium, but the titration was carried out in a one phase system.

In 1961, Hefferen and Dietz (33) proposed a potentiometric method for the analysis of benzethonium. In this

method the substance was reacted with potassium ferricyanide and the excess reagent was titrated with silver nitrate. Weiner and Felmeister (34) recently analysed cetylpyridium chloride by thermometric titration. The endpoint was detected by the enthalpy change which results when the compound is reacted with Orange II.

An ion exchange method for methantheline and propantheline was presented by Kracmar and Zyka (35). They utilized a column of Amberlite IRA-400 which gave them the free base of the compound, which was subsequently estimated by residual titration.

A gravimetric method for pentolinium tartrate involving the formation of the reineckate derivative is described in the B.P. (26).

STATEMENT OF PROBLEM

Quaternary ammonium compounds today constitute the active ingredient in a wide variety of pharmaceutical products. They are pharmacologically active as antiseptics, antifungals, cholinergics, anticholinergics, antispasmodics and antihypertensives. Despite the importance of these compounds a general method for their quantitative analysis is not yet available.

Although several methods have beem employed in the analysis of these substances, disadvantages were found to exist in all of them. The ultraviolet method is sensitive and rapid, however, interferences from tablet excipients occur in many instances. Volumetric methods have been employed but they require large samples and cannot be applied to many dosage forms. The gravimetric method also required large samples and is very time consuming.

It was the purpose of this investigation, therefore, to develop a sensitive and rapid method of analysis which could be applied to a large number of dosage forms whose active ingredient contains the quaternary ammonium functional group. A colourimetric method which involves complexation of the medicinal agents with various acid dyes was selected as a possible solution to the problem.

EXPERIMENTAL

Apparatus

Beckman Model B Spectrophotometer, Beckman Model DB Spectrophotometer, Beckman Zeromatic II, Mettler Gramatic Balance, Sargent Magnetic Stirrer and conventional laboratory glassware.

Chemicals and Reagents

The following is a list of all the chemicals used in the investigation: chloroform, methylene chloride, glacial acetic acid, ether, hydrochloric acid, potassium chloride, potassium biphthalate, sodium hydroxide, potassium phosphate and boric acid. All of the above chemicals were of A.C.S. reagent grade.

The following is a list of the reagents used: 0.0001N. methyl orange, 0.0001N. Orange IV, 0.0001N. Orange II, 0.0001N. bromphenol blue, 0.0001N. bromthymol blue (the above dye solutions were buffered with Clark and Lubs phosphate buffers), 0.05N. perchloric acid in dioxane (standardized against potassium acid phthalate), 0.02N. sodium tetraphenylboron, 6% mercuric acetate, crystal violet indicator (0.5% in glacial acetic acid), methyl red indicator (0.1% in glacial acetic acid) and bromphenol blue solution (1 in 2,000).

The following is a list of the quaternary ammonium substances used in this investigation:

1. Ambenonium Chloride (Winthrop Laboratories)
2. Benzethonium Chloride (Parke, Davis & Co. Ltd.)
3. Benthanecol Chloride (Merck, Sharp & Dohme of Canada Ltd.)
4. Cetylpyridinium Chloride (The Wm. S. Merrell Co.)

5. Chlorisondamine Chloride (Ciba Company Ltd.)
6. Demecarium Bromide (Merck, Sharp & Dohme of Canada Ltd.)
7. Domiphen Bromide (Ciba Company Ltd.)
8. Echothiophate Iodide (Ayerst Laboratories)
9. Edrophonium Bromide (Hoffman-LaRoche Ltd.)
10. Hexocyclium Methosulfate (Abbott Laboratories Ltd.)
11. Isopropamide Iodide (Smith, Kline & French)
12. Mepenzolate Bromide (Lakeside Laboratories Ltd.)
13. Methantheline Bromide (G.D. Searle & Co. of Canada Ltd.)
14. Oxphenonium Bromide (Ciba Company Ltd.)
15. Penthienate Bromide (Winthrop Laboratories)
16. Pentolinium Tartrate (Poulenc Limited)
17. Pipenzolate Bromide (Lakeside Laboratories)
18. Propantheline Bromide (G.D. Searle & Co. of Canada Ltd.)
19. Pyridostigmine Bromide (Hoffman-LaRoche Ltd.)
20. Trimethidinium Methosulfate (John Wyeth & Brother (Can.) Ltd.)
21. Valethamate Bromide (Ayerst Laboratories)

Procedures

I. Analysis of Crystalline Quaternary Ammonium Materials

All of the crystalline materials were assayed by non-aqueous titration.

Procedure A

Fifty to 100 mg. of the compound were accurately weighed into a 100 ml. beaker and dissolved in 25 ml. of glacial acetic acid with the aid of a magnetic stirrer. After the addition of 2 ml. mercuric acetate T.S., and 2 drops of crystal violet indicator, the solution was titrated with 0.05N. perchloric acid in dioxane to a blue endpoint. A blank titration was performed on the solvent system and the necessary corrections were made.

The following compounds were analysed by this procedure: penthienate bromide, isopropamide iodide, mepenzolate bromide, pipenzolate bromide, demecarium bromide, bethanecol chloride and pentolinium tartrate.

Procedure B

Fifty to 100 mg. of the compound were accurately weighed into a 100 ml. beaker and dissolved in 25 ml. of chloroform. Mercuric acetate T.S. (2 ml.) and 2 drops of methyl red indicator were added and then titrated with 0.05N. perchloric acid in dioxane to a pink endpoint. A blank was performed on the solvent system and the appropriate corrections were made.

The following compounds were analysed by this procedure: oxphenonium bromide, cetylpyridinium chloride, benzethonium chloride, methantheline bromide, propantheline bromide, domiphen bromide, hexocyclium methosulfate, pyridostigmine bromide and valethamate bromide.

Procedure C

This procedure is identical to procedure A except that methyl red was employed as the indicator instead of crystal violet. Edrophonium bromide was the only compound determined by this procedure.

II. Selection of Complexing Agent

A 0.0001N. solution was prepared from each dye and buffered at pH values of 2,4,6,8 and 10. Five millilitres of 0.0001N. benzethonium chloride were added to 10 ml. of the buffered dye solution in a 250 ml. separatory funnel. The complex was extracted with 10 ml. of chloroform and the lower organic layer was drawn into a cuvette. The cuvettes were stoppered and absorbance measurements were taken for 120 minutes at the optimum wavelength.

III. Determination of Optimum Wavelength

A suitable volume of a 0.05 mg./ml. solution of the quaternary ammonium compound was added to 10 ml. of the 0.0001N. solution of the complexing dye in a 250 ml. separatory funnel. Twenty-five millilitres of methylene chloride were added and the funnel was shaken vigorously for 1 minute.

The system was allowed to equilibrate for 10 minutes and the lower organic layer was drawn into a cuvette. Absorbance readings were taken over the visible range and the optimum wavelength determined. This wavelength was used for all subsequent determinations of that particular compound under the same conditions.

IV. Calibration Curves

General Procedure

Fifty milligrams of the quaternary ammonium compound were accurately weighed into a 100 ml. volumetric flask and dissolved in distilled water. This was made up to volume with distilled water and 10 ml. of this solution was diluted to 100 ml. to produce a 0.05 mg./ml. solution.

To five 250 ml. separatory funnels, each containing 10 ml. of a 0.0001N. solution of the dye, were added 1,2,3,4 and 5 ml. of the 0.05 mg./ml. solution of the quaternary ammonium compound. Sufficient distilled water was added to make the aqueous volume constant in each separatory funnel. Twenty-five millilitres of methylene chloride were then added from a 50 ml. burette. The separatory funnel was shaken vigorously for 1 minute and the system allowed to equilibrate for 10 minutes. The inside of the funnel stem was wiped clean with absorbant paper. The organic phase was then drawn into a cuvette and the absorbance was measured at the optimum wavelength, using a methylene chloride blank. The procedure was repeated 5 times and the calibration curve was constucted

from the average.

Procedure A

The general procedure was employed with methyl orange at pH 8.0 as the complexing dye. This procedure was used for the following compounds: benzethonium chloride, cetylpyridinium chloride, domiphen bromide, hexocyclium methosulfate, isopropamide iodide, methantheline bromide, oxyphenonium bromide and propantheline bromide. For cetylpyridinium chloride and propantheline bromide only 20 ml. of methylene chloride was used to extract the complex.

Procedure B

This procedure, which employed Orange IV as the complexing agent, was followed for the calibration curves of ambenonium chloride, demecarium bromide, penthienate bromide, pipenzolate bromide, and mepenzolate bromide. The technique was basically that of the general procedure with slight modifications. The general procedure was followed directly for mepenzolate and pipenzolate. For ambenonium the complexing dye was 20 ml. of Orange IV at pH 6.0. The equilibration time, however, was 30 minutes and the organic phase was drawn into a 25 ml. volumetric flask and the contents shaken. A portion of this was added to a cuvette and the absorbance was measured at 410 mμ.

Demecarium required a 20 minute equilibration time and also 10 minutes to stabilize the colour in the cuvette before absorbance measurements could be taken. The general

procedure was followed for penthienate with the only modification being a 15 minute allowance for colour development before the absorbance was measured.

Procedure C

The general procedure was employed with 20 ml. of bromthymol blue at pH 7.0 as the complexing agent. This procedure was employed for the following compounds: echothiophate iodide, edrophonium bromide, pentolinium tartrate, pyridostigmine bromide and trimethidinium methosulfate.

V. Analysis of Pharmaceutical Dosage Forms

1. General Procedure for Tablets

Ten tablets were weighed and finely powdered. A sample of powder equivalent to 5 mg. of the quaternary ammonium compound was weighed into a 150 ml. beaker and stirred magnetically for 15 minutes with 40 ml. distilled water. This solution was suction filtered through Whatman No. 1 paper into a 125 ml. flask. The beaker and residue were washed with a further 30 ml. of distilled water and the filtrate was made up to volume in a 100 ml. volumetric flask. Three millilitres of this solution (theoretically 0.05 mg./ml.) and 2 ml. of distilled water were added to the dye solution employed in the preparation of its calibration curve. The remainder of the procedure was identical to that described for the calibration curve of each particular substance.

2. General Procedure For Injections and Solutions

A suitable volume of the injection was diluted to yield a theoretical concentration of 0.05 mg./ml. Three millilitres of this solution were added to the appropriate dye solution. The remainder of the procedure was identical to that described for the calibration curve of each particular substance.

3. Dosage Forms of Ambenonium Chloride

Both Mytelase Tablets 10 and 25 mg., were assayed by the general procedure for tablets.

4. Dosage Forms of Benzethonium Chloride

a) Phemerol Chloride Solution 1:1,000 was assayed by the general procedure for injections and solutions.

b) Phemerol Chloride Tincture 1:500. Ten millilitres of the tincture were pipetted into a 150 ml. beaker containing 10 ml. of distilled water. This solution was heated on a water bath to evaporate the acetone and alcohol. After cooling, this solution was made up to 100 ml. in a volumetric flask. Twenty-five millilitres of the resulting solution were diluted to 100 ml., yielding a theoretical concentration of 0.05 mg./ml. of benzethonium. Three millilitres of this solution and 2 ml. of distilled water were added to 10 ml. of 0.0001N. methyl orange. The remainder of the procedure was identical to that described for the calibration curve beginning with "Twenty-five millilitres of methylene chloride ---".

5. Dosage Forms of Cetylpyridinium Chloride

a) Cepacol Solution 1:2,000. Ten millilitres of the solution were pipetted into a 150 ml. beaker and heated over a steam bath to evaporate the ethanol. The solution was cooled to room temperature, then made up to 100 ml. in a volumetric flask. Three millilitres of this solution and 2 ml. of distilled water were added to 10 ml. of 0.0001N. methyl orange in a 250 ml. separatory funnel. The remainder of the procedure was identical to that described for the calibration curve beginning with "Twenty-five millilitres of methylene chloride ---".

b) Cepacol Lozenges. Five lozenges were weighed and finely powdered. A sample equivalent to 2.5 mg. of cetylpyridinium chloride was weighed in a beaker and stirred magnetically for 30 minutes with 25 ml. distilled water. The solution was made up to volume in a 50 ml. volumetric flask. Three millilitres of this solution and 2 ml. of distilled water were added to 10 ml. of 0.0001N. methyl orange at pH 8.0 in a separatory funnel. The remainder of the procedure was identical to that described for the calibration curve beginning with "Twenty-five millilitres of methylene chloride ---". A blank was also determined replacing the dye solution with 10 ml. of distilled water.

6. Dosage Forms of Demecarium Bromide

Humorsol Ophthalmic Solution 0.25% was assayed by the general procedure for solutions and injections.

7. Dosage Forms of Domiphen Bromide

a) Bradosol Lozenges were assayed by the general procedure for tablets but the stock solution was made up to a theoretical concentration of 0.03 mg./ml.

b) Bradosol Powder was assayed by the procedure used for the domiphen bromide calibration curve.

8. Dosage Forms of Echothiophate Iodide

Phospholine Iodide 3.0, 6.25 and 12.5 mg. The contents of the vial were dissolved in distilled water and transferred to a 100 ml. volumetric flask, then made up to volume with distilled water. An appropriate quantity (whose absorbance would fall within the limits of the calibration curve) was added to a separatory funnel containing 20 ml. of 0.0001N. bromthymol blue at pH 7.0. The remainder of the procedure was identical to that described for the calibration beginning with "Twenty-five millilitres of methylene chloride ---".

9. Dosage Forms of Edrophonium Bromide

Tensilon Injection was assayed by the general procedure for injections and solutions, but a solution with a theoretical concentration of 0.5 mg./ml. was initially prepared. This was then diluted to 0.05 mg./ml.

10. Dosage Forms of Hexocyclium Methosulfate

Tral with Phenobarbital Tablets were assayed by the general procedure for tablets except that a sample equivalent

to 50 mg. of hexocyclium was employed. After filtration etc. a solution of theoretical concentration 0.05 mg./ml. was prepared.

11. Dosage Forms of Isopropamide Iodide

Darbid Tablets 5 mg. were assayed by the general procedure for tablets.

12. Dosage Forms of Methantheline Bromide

Banthine Tablets 50 mg. were assayed by the general procedure for tablets.

13. Dosage Forms of Oxyphenonium Bromide

Antrenyl Tablets, 5 and 10 mg., were analysed by the general procedure for tablets.

14. Dosage Forms of Penthienate Bromide

Monodral Tablets 5 mg. were analysed by the general procedure for tablets and Monodral Elixir followed the general procedure for injections and solutions.

15. Dosage Forms of Pentolinium Tartrate

a) Ansolysen Tablets 40 mg. The general procedure for tablets was used except that it was first required to prepare a solution of theoretical concentration 0.5 mg./ml.

b) Ansolysen Injection. Five millilitres of the injection were pipetted into a 250 ml. volumetric flask and made up to volume with distilled water. To 20 ml. of 0.0001N. bromthymol blue at pH 7.0, were added 1.5 ml. of

the diluted Ansolysen Injection and 3.5 ml. of distilled water. The remainder of the procedure was identical to that described for the calibration curve beginning with "Twenty-five millilitres of methylene chloride ---".

16. Dosage Forms of Pipenzolate Bromide

Piptal Tablets 5 mg. were assayed by the general procedure for tablets.

17. Dosage Forms of Propantheline Bromide

a) Pro-Banthine Tablets 7.5 mg. Ten tablets were weighed and finely powdered. Fifty milligrams of the powdered material were weighed in a 150 ml. beaker and stirred magnetically for 30 minutes with 25 ml. of methylene chloride. The solution was filtered through Whatman No. 1 paper and the residue was washed with a further 10 ml. of methylene chloride. The filtrate was evaporated to dryness and the residue was dissolved in water and made up to volume in a 100 ml. volumetric flask. The remainder of the procedure was identical to that described for its calibration curve.

b) Pro-Banthine Tablets 15 mg. The procedure was identical to that described for the 7.5 mg. tablets except that only 25 mg. of the powdered tablets were used.

c) Pro-Banthine Tablets 30 mg. The general procedure for tablets was used.

d) Pro-Banthine Injection. The contents of the ampoule were transferred to a beaker and weighed. Distilled water was added to dissolve the powder and the solution was

transferred to a 100 ml. volumetric flask and made up to volume. Appropriate dilutions were made to ensure that the absorbance would fall within the limits of the calibration curve. The remainder of the procedure is identical to that described for its calibration curve.

18. Dosage Forms of Pyridostigmine Bromide

Mestinon Tablets 60 mg. were assayed by the general procedure except that a sample equivalent to 50 mg. of pyridostigmine was used and from this appropriate dilutions were made.

19. Dosage Forms of Trimethidinium Methosulfate

Ostensin Tablets 20 mg. were assayed by the general procedure for tablets.

RESULTS AND DISCUSSION

Analysis of Crystalline Quaternary Ammonium Materials

The results for the analysis of crystalline materials are presented in Table I.

TABLE I. ANALYSIS OF CRYSTALLINE QUATERNARY AMMONIUM MATERIALS

Name of Compound	% Purity
1. Benzethonium Chloride	99.9
2. Bethanecol Chloride	101.1
3. Cetylpyridinium Chloride	99.9
4. Demecarium Bromide	101.8
5. Domiphen Bromide	99.5
6. Edrophonium Bromide	100.1
7. Hexocyclium Methosulfate	99.5
8. Isopropamide Iodide	98.9
9. Mepenzolate Bromide	100.6
10. Methantheline Bromide	99.9
11. Oxyphenonium Bromide	100.7
12. Penthienate Bromide	98.7
13. Pentolinium Tartrate	98.9
14. Pipenzolate Bromide	99.4
15. Propantheline Bromide	97.0
16. Pyridostigmine Bromide	99.5
17. Valethamate Bromide	100.9

In all instances the pure compound was analysed by non-aqueous titration. The figures reported are the average of three determinations. Crystal violet was found to be a more suitable indicator for those compounds soluble in acetic acid and methyl red was more suitable for the chloroform soluble substances.

Satisfactory results were obtained for all compounds except ambenonium chloride, chlorisondamine chloride, echothiophate iodide and trimethidinium methosulfate. Endpoints could not be reached for these medicinal agents. Alternative methods of analysis were not found in the literature so melting points were taken for the latter four compounds in order to determine their purity. These results are shown in Table II.

TABLE II. MELTING POINTS OF CRYSTALLINE MATERIALS

Compound	Reported M.P. (°C.)	Observed M.P. (°C)
Ambenonium Chloride	196-199	196-198
Chlorisondamine Chloride	258-265 (decomp.)	259-263
Echothiophate Iodide	116-122 (decomp.)	116-120
Trimethidinium Methosulfate	192-193	198-199

Of the substances which did not give results by non-aqueous titration only echothiophate is not a bisquaternary compound. A study by Helgren, Theivagt and Campbell (11) on hexocyclium methosulfate showed that non-aqueous titration

involved the non-quaternized nitrogen of the piperazine ring. They made their observations on the fact that the completely quaternized derivative of hexocyclium would not react with perchloric acid in acetic acid. They concluded that the methyl sulfate anion is a weaker base than the acetate ion and therefore would not titrate. This may be the explanation for the failure of trimethidinium methosulfate to titrate. Explanations are not available for ambenonium and chlorisondamine because other bisquaternary ammonium compounds such as demecarium and pentolinium gave good results by non-aqueous titration. Studies on echothiophate (36) have shown that the compound undergoes degradation which may explain the anomalous results obtained with this substance.

Determination of Optimum Conditions and Suitable Reagents

Before discussing the reagents and conditions selected for this investigation, it would be meaningful to present the theory of the dye partition technique.

Organic liquids such as chloroform or methylene chloride are not good solvents for ionic dyes. Therefore, in a two phase system when one such immiscible solvent is present with an aqueous solution of a dye or indicator, little or no colour is transferred to the organic phase. However, when a quaternary ammonium compound is present along with an excess of the anionic dye, the complex which is formed is partitioned preferentially in the organic phase.

Helgren, Theivagt and Campbell (11) have summarized the reactions in the following manner.

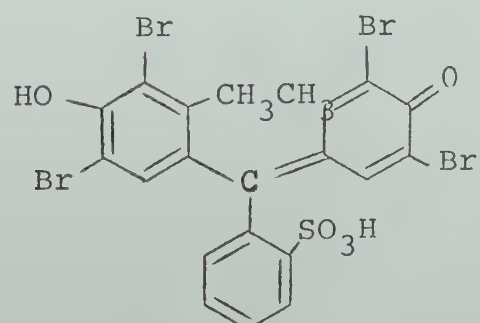
$$\begin{array}{ccccc} \mathrm{DNa}\ (H_2O) & \rightleftharpoons & \mathrm{RD}(H_2O) & \rightleftharpoons & \mathrm{RX}(H_2O) \\ \updownarrow K_2 & & \updownarrow K_1 & & \updownarrow K_3 \\ \mathrm{DNa}\ (\mathrm{org.}) & \rightleftharpoons & \mathrm{RD}(\mathrm{org.}) & \rightleftharpoons & \mathrm{RX}(\mathrm{org.}) \end{array}$$

The anionic dye is represented by DNa, the cationic compound by RX, and the complex RD. The equilibria K_2 and K_3 represent the partitioning of the dye and the quaternary ammonium compound respectively between water and the organic phase, while K_1 represents the partitioning of the complex between the two phases. In order for the method to succeed K_1 must favor the organic phase.

Preliminary studies for the selection of a suitable dye were made with benzethonium chloride. The dyes chosen for this study were bromphenol blue, bromthymol blue, Orange IV, Orange II and methyl orange. Their structures are given below.

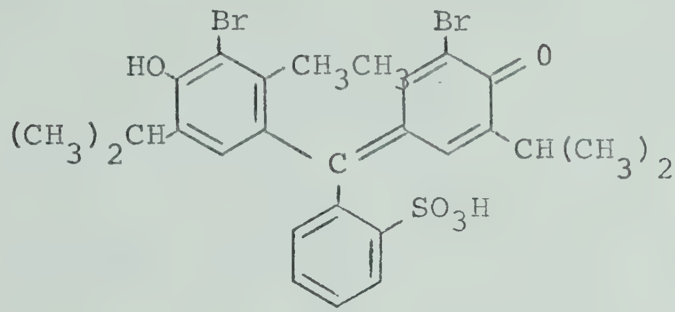
-Bromphenol blue (3,3',5,5'-Tetrabromophenol sulfonphthalein)

Molecular weight - 670.02



-Bromthymol blue (3,3'-Dibromothymol sulfonphthalein)

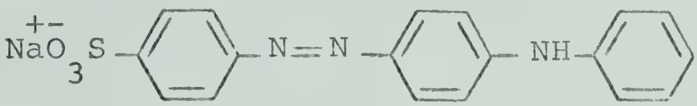
Molecular weight - 624.39



-Orange IV (Tropaeolin 00)

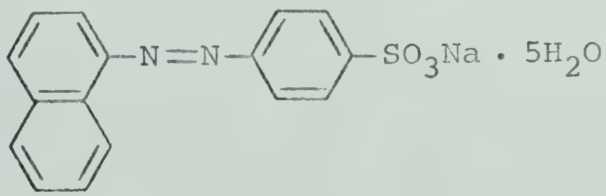
(Sodium p-dimethylamine-azobenzene sulfonate)

Molecular weight - 375.88



-Orange II (p-(2-hydroxy-1-naphthylazo) benzene sulfonic acid)

Molecular weight - 440.41



-Methyl Orange (sodium p-dimethylaminobenzene sulfonate)

Molecular weight - 327.34

The results of this preliminary investigation are shown in Figures 1-5 and indicate that the absorbance of the complex is not a direct function of pH. Although the absorbance of the complex was generally greater at higher pH values, the complexes of methyl orange, Orange IV, Orange II and bromphenol blue also showed high absorbance at pH 2.0. It is difficult to explain this phenomenon because at pH 2.0 the acid dye would be in the undissociated form and thus hinder complexation.

In most instances the optimum wavelength was the same for all complexes involving one particular dye. The complexes of bromphenol blue at pH 8.0 and 10.0 were blue, however, at pH 2.0, 4.0 and 6.0 the complexes showed the usual yellow colour. The complexes at pH 8.0 and 10.0 were observed to be very unstable. On the other hand methyl orange was found to give complexes with high absorbances and they exhibited the greatest stability of any in this study. Orange IV gave fairly stable complexes but the absorbances were lower than those of the methyl orange complexes. Although bromthymol blue complexes gave high absorbances at certain pH values, they were found to be much more pH dependent. Orange II complexes were generally unstable and gave lower absorbances than those formed with other dyes.

FIGURE 1. STABILITY OF BENZETHONIUM-METHYL ORANGE COMPLEXES

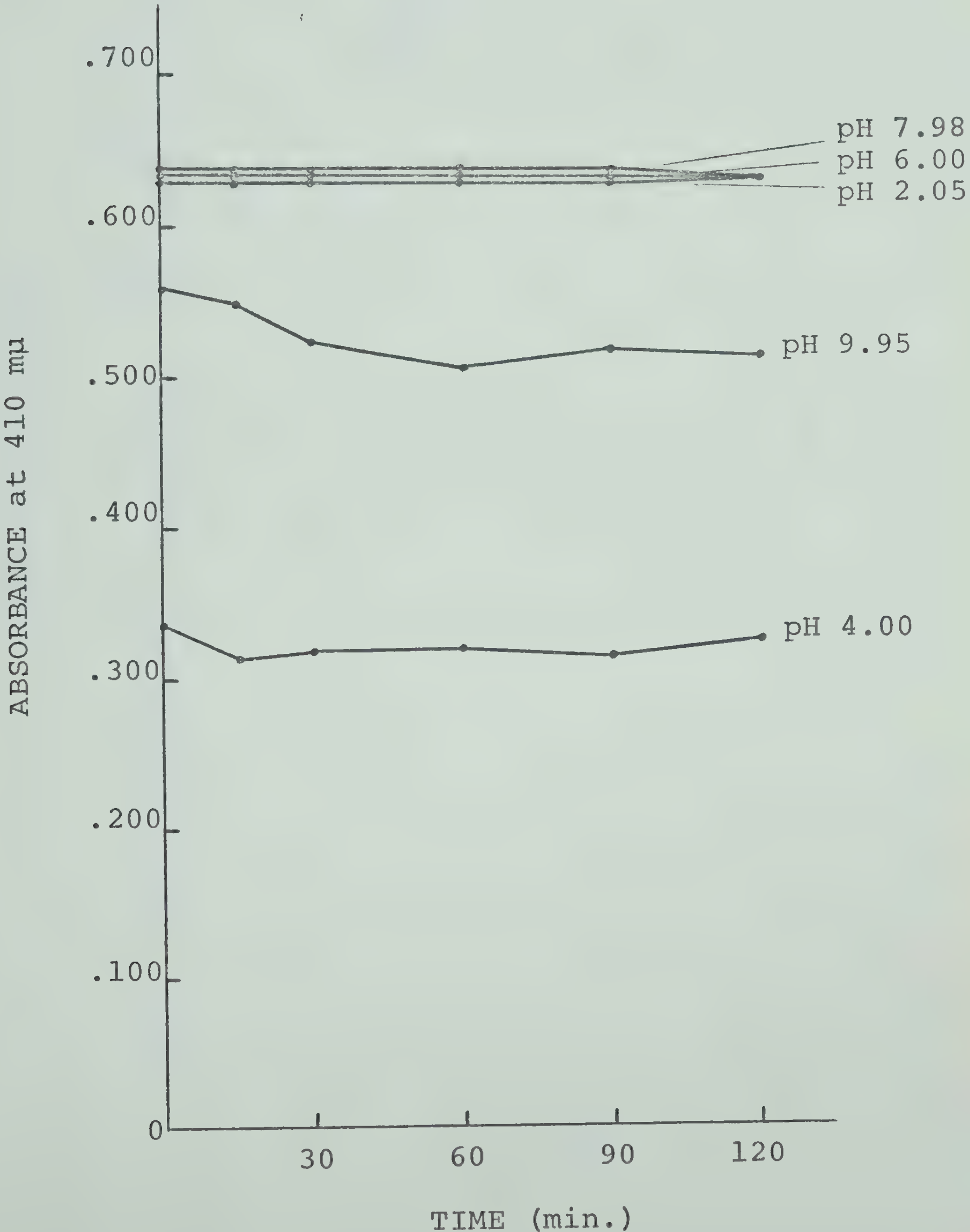


FIGURE 2. STABILITY OF BENZETHONIUM-ORANGE IV COMPLEXES

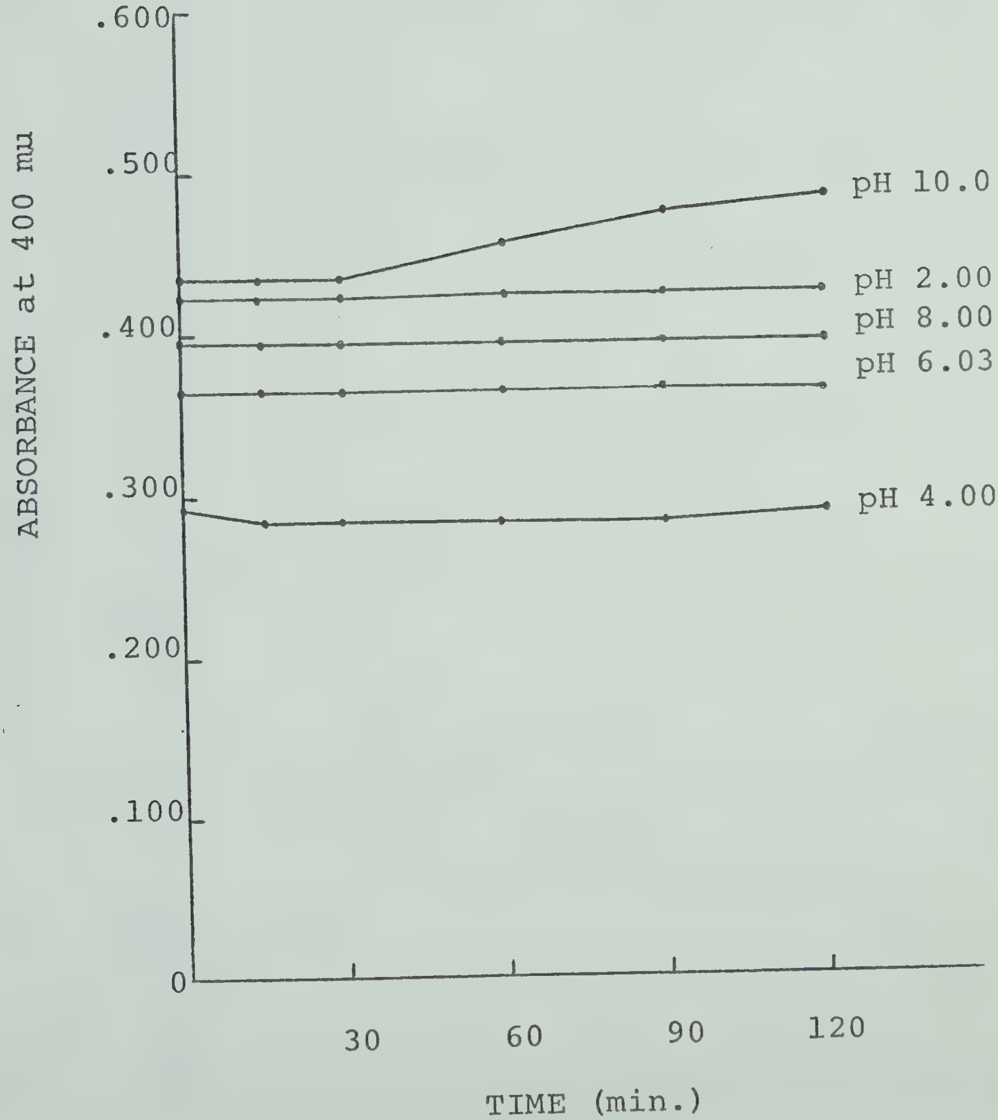


FIGURE 3. STABILITY OF BENZETHONIUM-BROMTHYMOL BLUE COMPLEXES

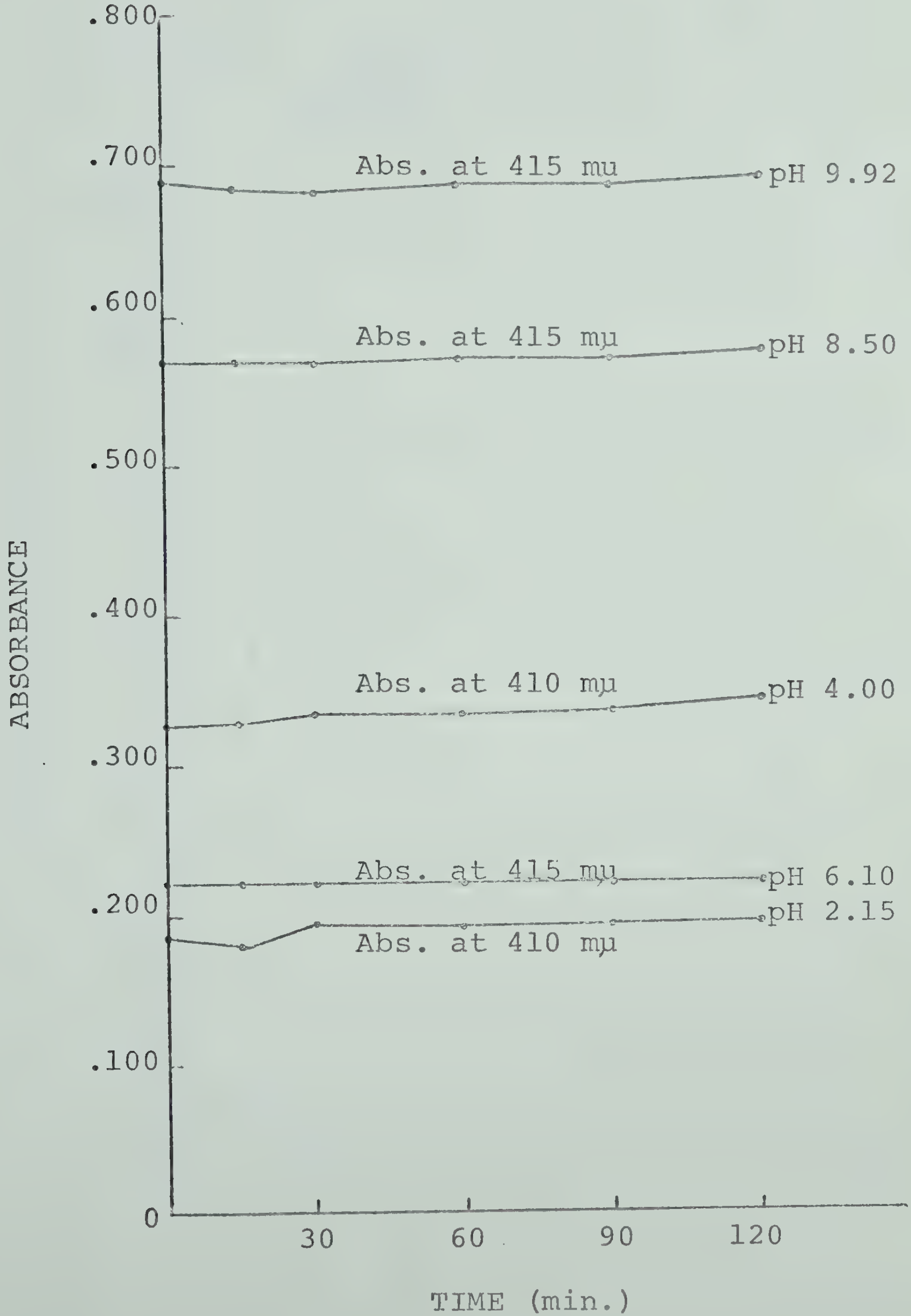


FIGURE 4. STABILITY OF BENZETHONIUM ORANGE II COMPLEXES

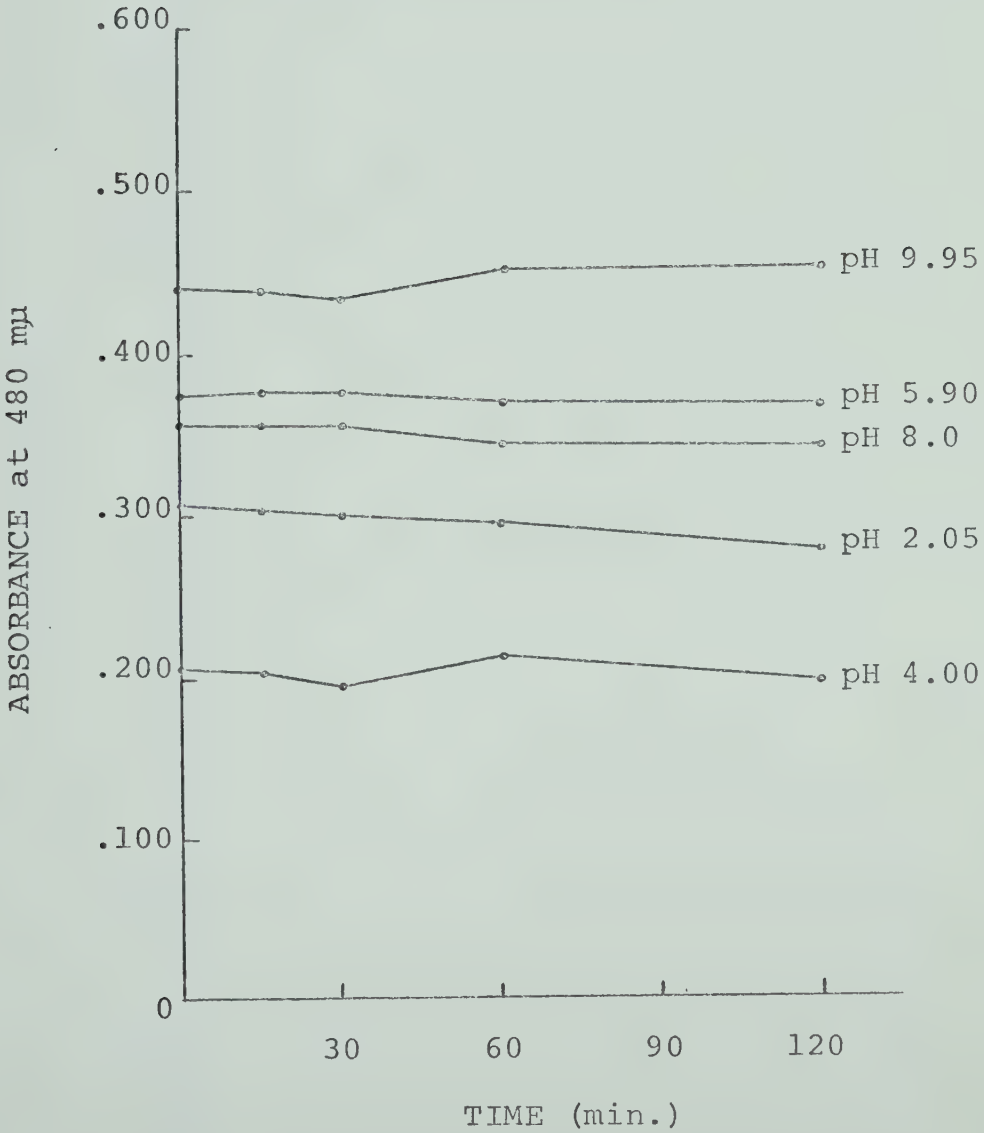
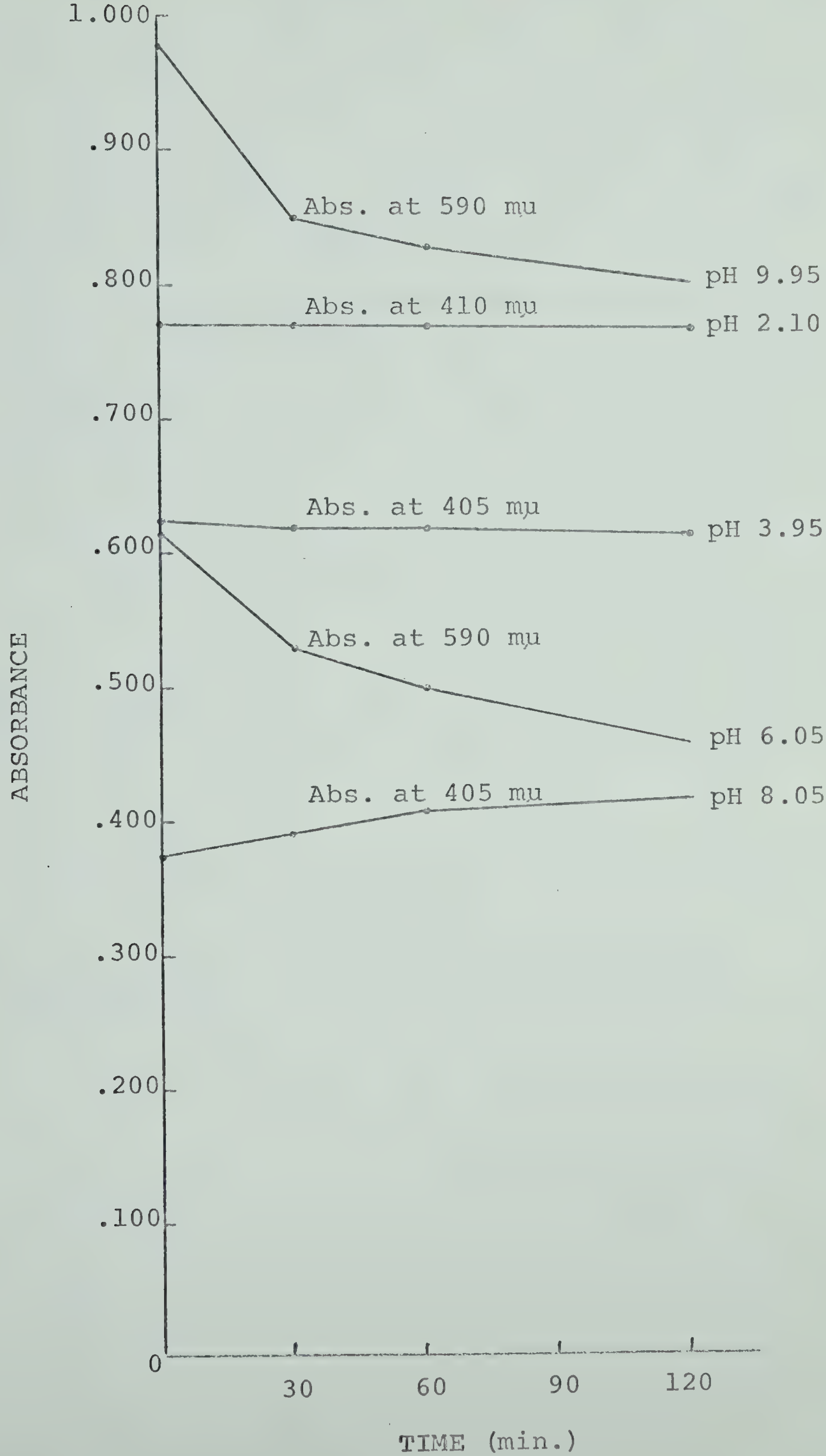


FIGURE 5. STABILITY OF BENZETHONIUM-BROMPHENOL BLUE COMPLEXES



Although chloroform was used as the extracting solvent in the aforementioned study, methylene chloride was later found to be a more suitable solvent. In order to determine the amount of methylene chloride required to extract the complex, 5 ml. of a 0.0001N. solution of the dye were added to a 250 ml. separatory funnel. An excess of the quaternary ammonium compound was added to ensure a complete reaction of the dye. The complex was then extracted with methylene chloride. A clear aqueous phase indicated complete extraction by the organic solvent. In most instances 25 ml. was found to be a sufficient volume of methylene chloride.

Blank determinations were also performed on the dye alone. Varying quantities of a 0.0001N. solution of the dye were extracted with 25 ml. of methylene chloride and the absorbance of the organic layer was measured. The results of this investigation are given in Tables III-V.

TABLE III ABSORBANCE OF BROMTHYMOL BLUE AT pH 7.0

ml. of 0.0001N Bromthymol blue	Absorbance at 415 mμ
10	0.017
12	0.013
14	0.016
16	0.020
18	0.020

TABLE IV. ABSORBANCE OF METHYL ORANGE AT pH 8.0

ml. of 0.0001N. Methyl Orange	Absorbance at 410 mμ
5	0.002
7	0.000
9	0.000

TABLE V. ABSORBANCE OF ORANGE IV AT pH 6.0

ml. of 0.0001N. Orange IV	Absorbance at 400 mμ
5	0.000
7	0.000
9	0.001

These studies indicated that methyl orange was a satisfactory complexing agent at pH 8.0 because it gave very stable complexes with high absorbances, and the dye alone gave negligible absorbance when extracted with methylene chloride. By a similar series of experiments Orange IV and bromthymol blue were later found to be more suitable complexing agents for certain quaternary salts.

When the appropriate complexing dye for each quaternary compound was selected, the optimum wavelength for each complex was determined. These results are presented in Table VI. It was found that the optimum wavelength for all compounds complexed with a certain dye did not vary by more than 5 or 10 mμ. This would indicate that the quaternary

TABLE VI. DETERMINATION OF OPTIMUM WAVELENGTH

Drug	Complexing Dye	pH	Absorbances at Various Wavelengths 395	400	405	410	415	420	425
Ambenonium	Orange IV	6.0		.540	.580	.582*	.575	.550	.520
Benzethonium	Me. Orange	8.0		.600	.640	.680	.695*	.690	.680
Cetylpyridinium	Me. Orange	7.0	.860	.900	1.200*	1.100	1.000	.980	.950
Chlorisondamine	B.T.B.	≈6.0		.106	.106	.108	.110*	.105	.095
Dececarium	Orange IV	10.0		.675	.705	.730	.735*	.700	.660
Domiphen	Me. Orange	8.0		.144	.152	.157	.170*	.165	.160
Echothiophate	B.T.B.	7.0		.305	.315	.318*	.310	.300	.280
Edrophonium	B.T.B.	7.0		.640	.650	.660*	.650	.630	.595
Hexocyclium	Me. Orange	8.0		.290	.300	.310*	.300	.290	.270
Isopropamide	Me. Orange	8.0		.103	.109	.115	.116*	.112	.108
Mepenzolate	Orange IV	8.0	.180	.182*	.182	.178	.178		
Methantheline	Me. Orange	8.0		.530	.560	.580	.595*	.590	.575
Oxyphenonium	Me. Orange	8.0		.263	.277	.278	.283*	.280	.272
Penthienate	Orange IV	6.0	.545	.565	.578*	.578	.545	.495	.470
Pentolinium	B.T.B.	7.0		.770	.790	.800*	.780	.750	.700

TABLE VI. ...Continued

Drug	Complexing Dye	pH	395	Absorbances at Various Wavelengths 400	405	410	415	420	425
Pipenzolate	Orange IV	8.0		.650	.660	.670*	.660	.640	.600
Propantheline	Me. Orange	8.0		.630	.660	.660*	.650	.620	
Pyridostigmine	B.T.B.	7.0		.535	.535	.540*	.530	.510	.480
Trimethidinium	B.T.B.	7.0		.635	.660	.660*	.640	.620	.580

B.T.B. - Bromthymol Blue

Me. Orange - Methyl Orange

* - Optimum Wavelength

ammonium compound has little, if any effect on the absorbance of the complex.

Calibration Curves

Linear calibration curves are illustrated in Figures 6-24 for all pure compounds except bethanecol and mepenzolate. Preparation of all calibration curves was initially attempted using methyl orange as the complexing agent at pH 8.0. If the complex gave insufficient absorbance, Orange IV and then bromthymol blue were investigated as complexing agents.

For demecarium bromide, a stability study was required because absorbance readings of the solution increased on standing in a cuvette. The following are the results for the stability of the extracted complex in methylene chloride.

TABLE VII. STABILITY OF DEMECARIUM-ORANGE IV COMPLEX

Time (min.)	Abs.	Time (min.)	Abs.
1	.303	9	.378
2	.333	10	.378
3	.347	11	.381
4	.352	12	.381
5	.370	13	.381
6	.373	14	.381
7	.375	15	.383
8	.377	20	.385

FIGURE 6. CALIBRATION CURVE FOR

AMBENONIUM CHLORIDE

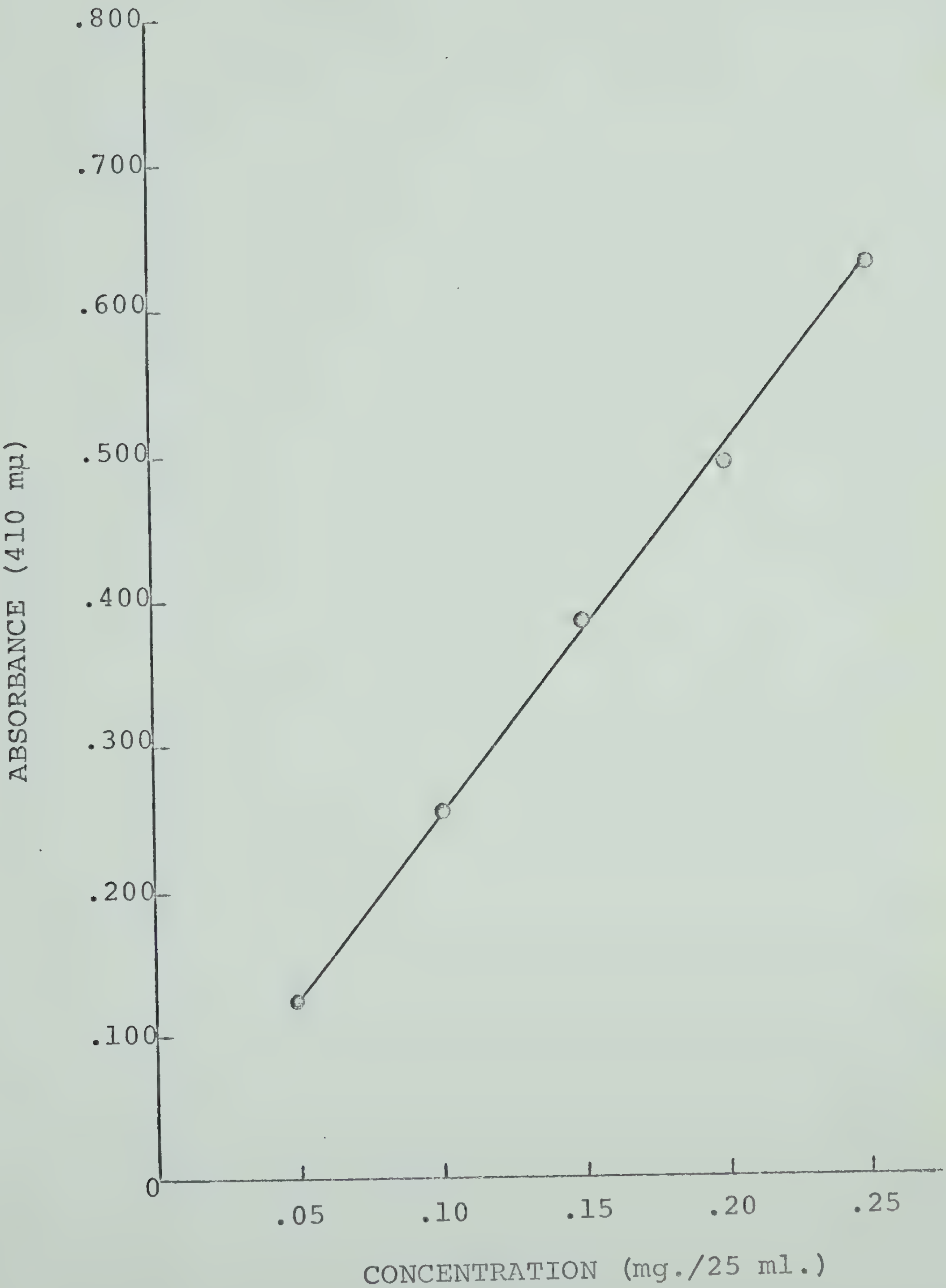


FIGURE 7. CALIBRATION CURVE FOR

BENZETHONIUM CHLORIDE

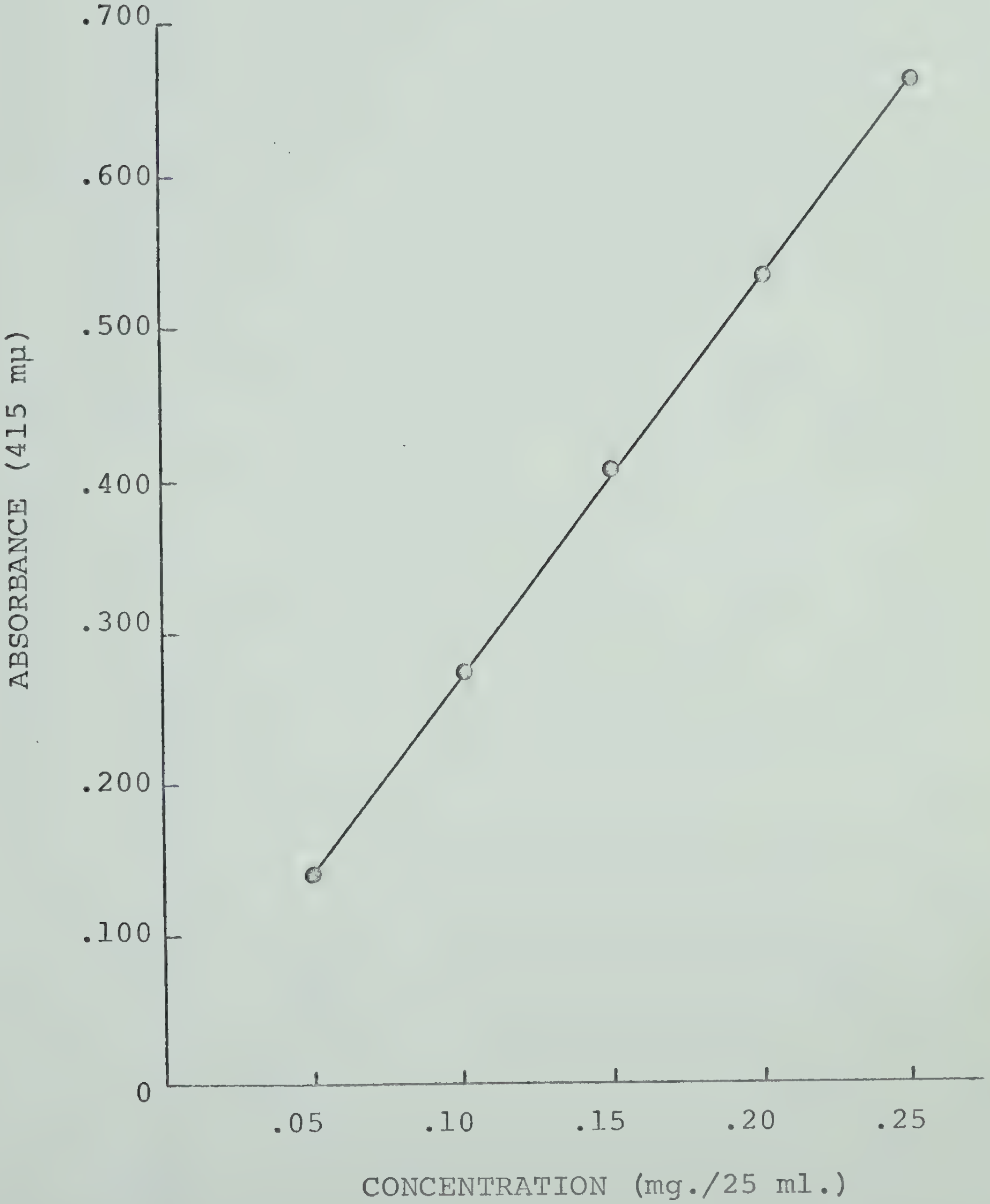


FIGURE 8. CALIBRATION CURVE FOR CETYLPYRIDINIUM CHLORIDE

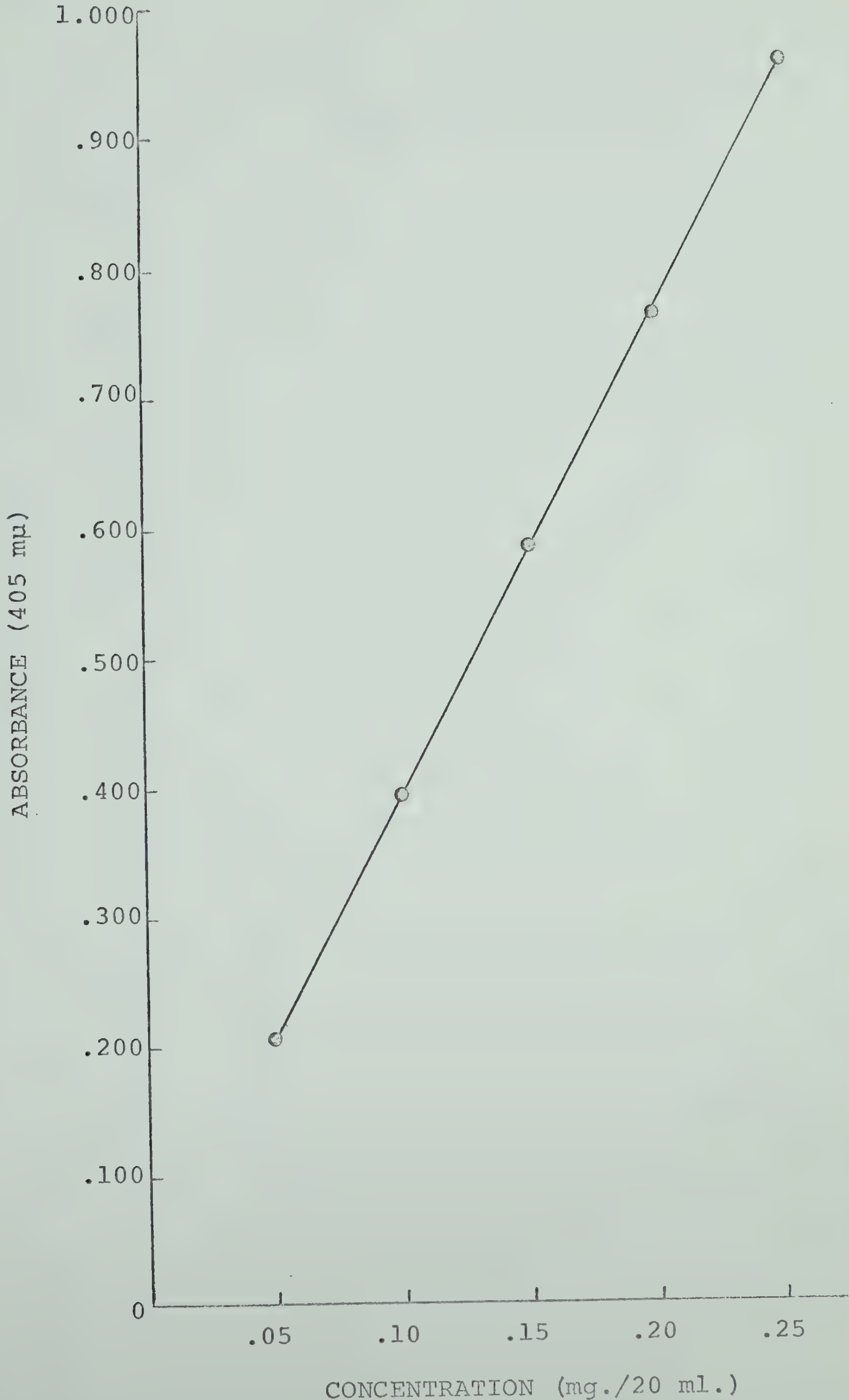


FIGURE 9. CALIBRATION CURVE FOR

CHLORISONDAMINE CHLORIDE

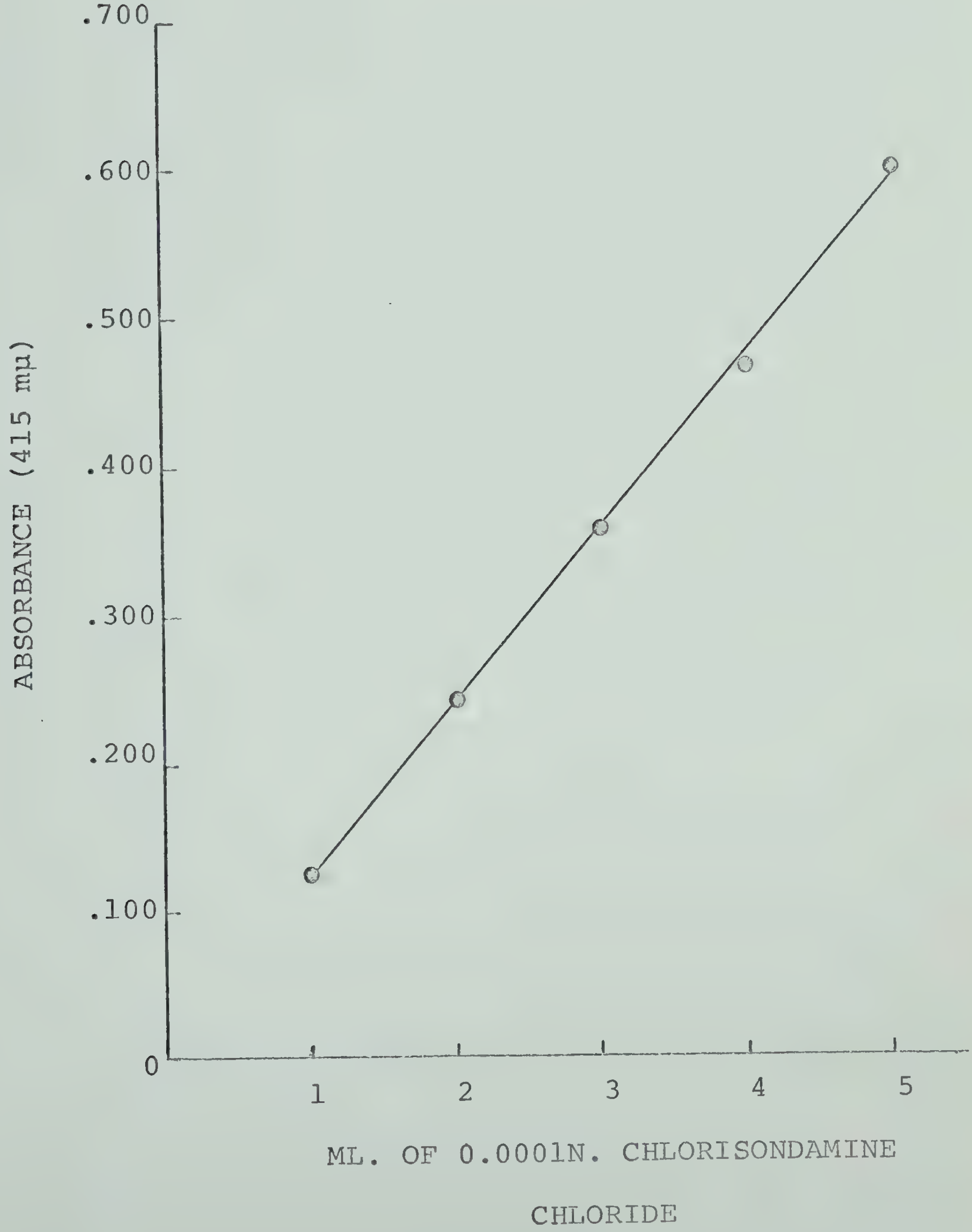


FIGURE 10. CALIBRATION CURVE FOR

DEMECARIUM BROMIDE

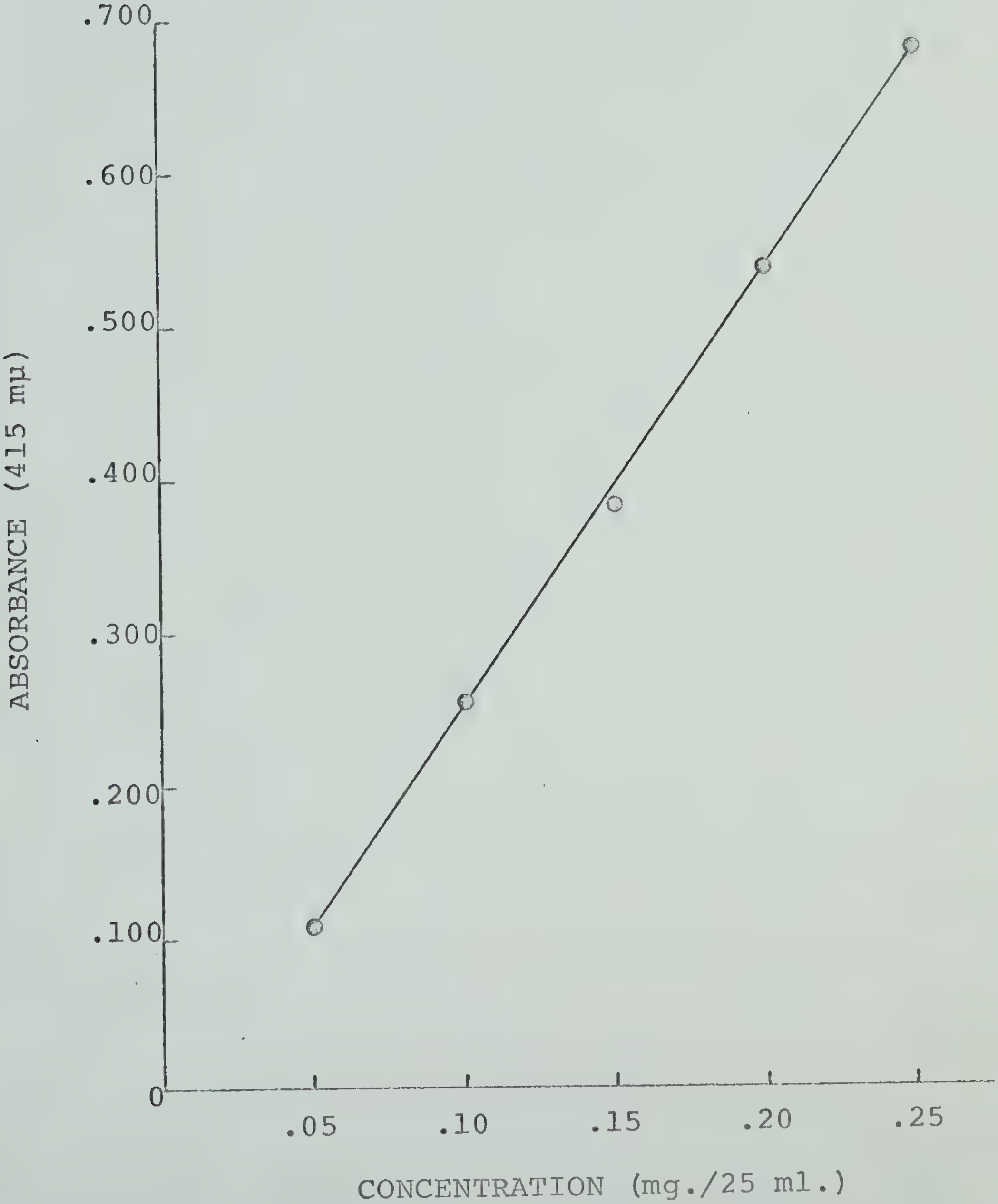


FIGURE 11. CALIBRATION CURVE FOR
DOMIPHEN BROMIDE

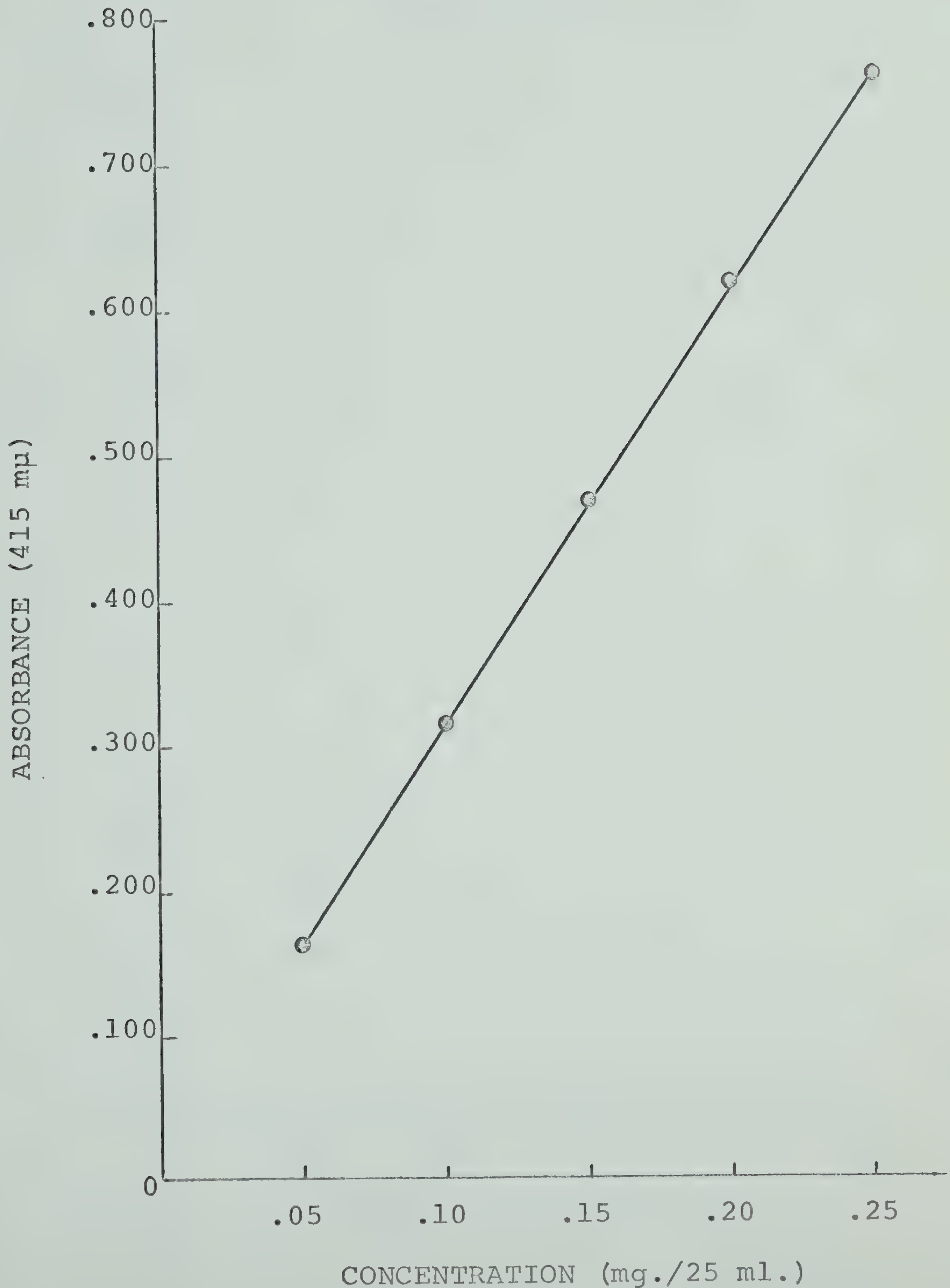


FIGURE 12. CALIBRATION CURVE FOR ECHOTHIOPHATE IODIDE

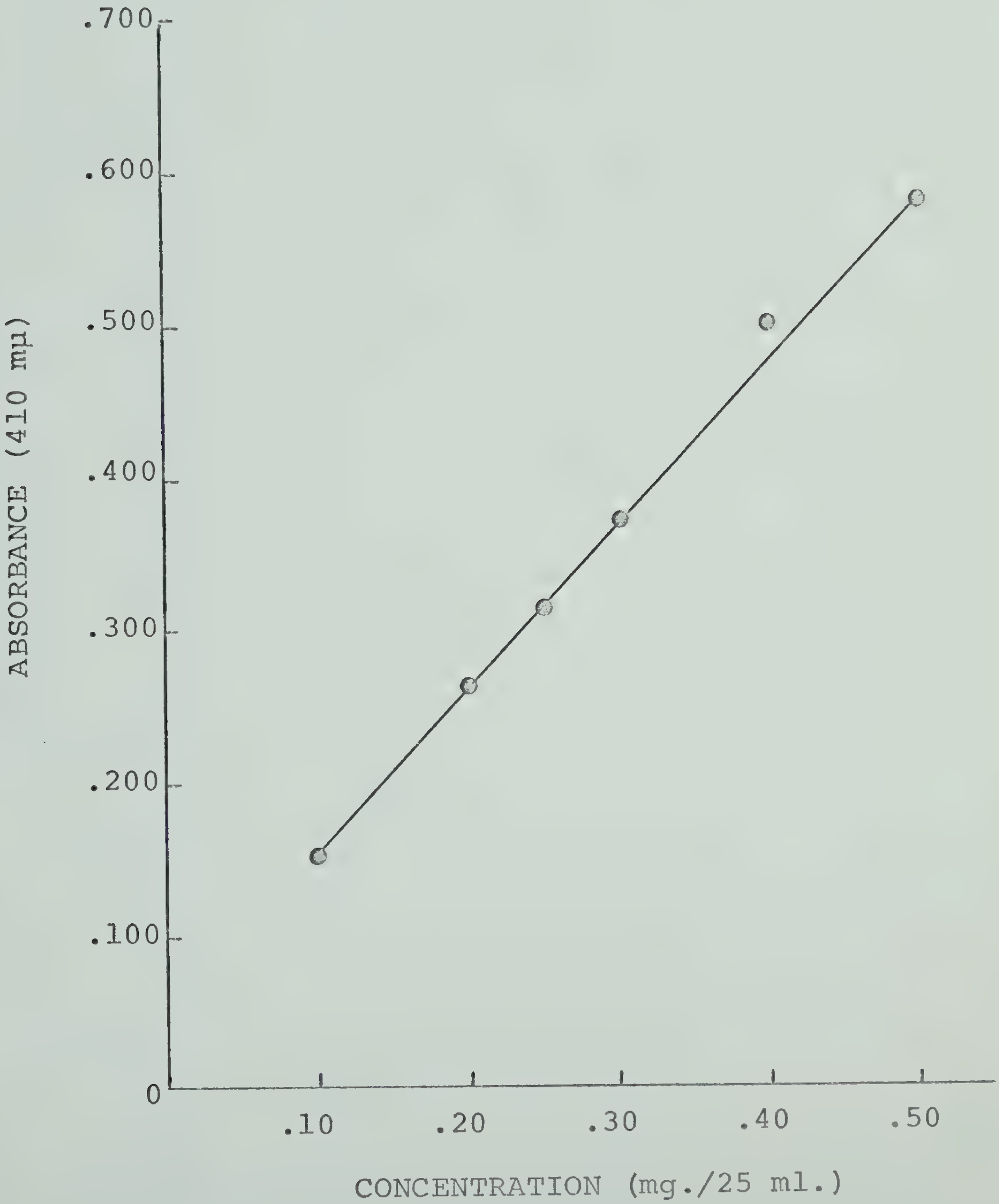


FIGURE 13. CALIBRATION CURVE FOR

EDROPHONIUM BROMIDE

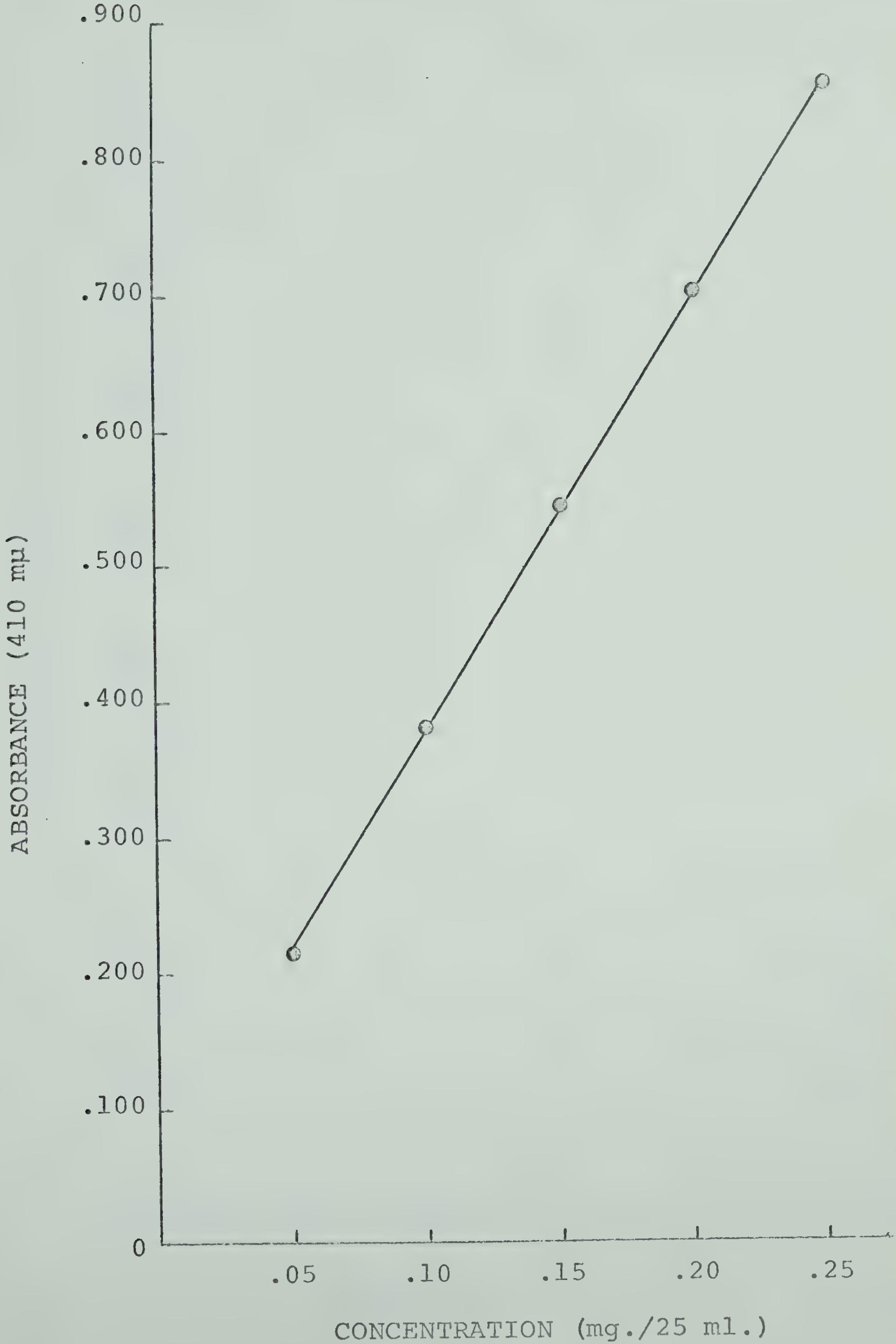


FIGURE 14. CALIBRATION CURVE FOR HEXOCYCLIUM METHOSULFATE

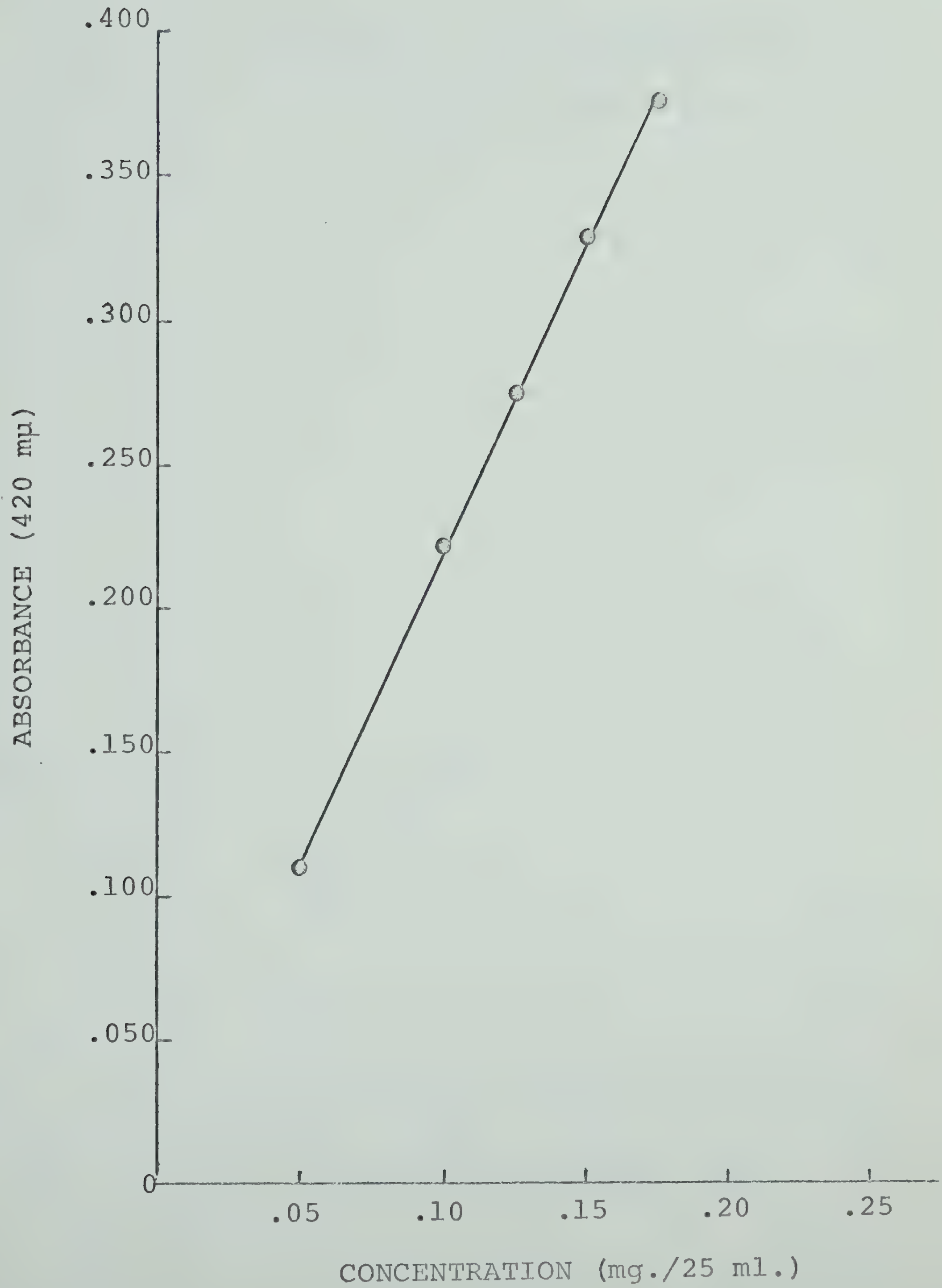


FIGURE 15. CALIBRATION CURVE FOR ISOPROPAMIDE IODIDE

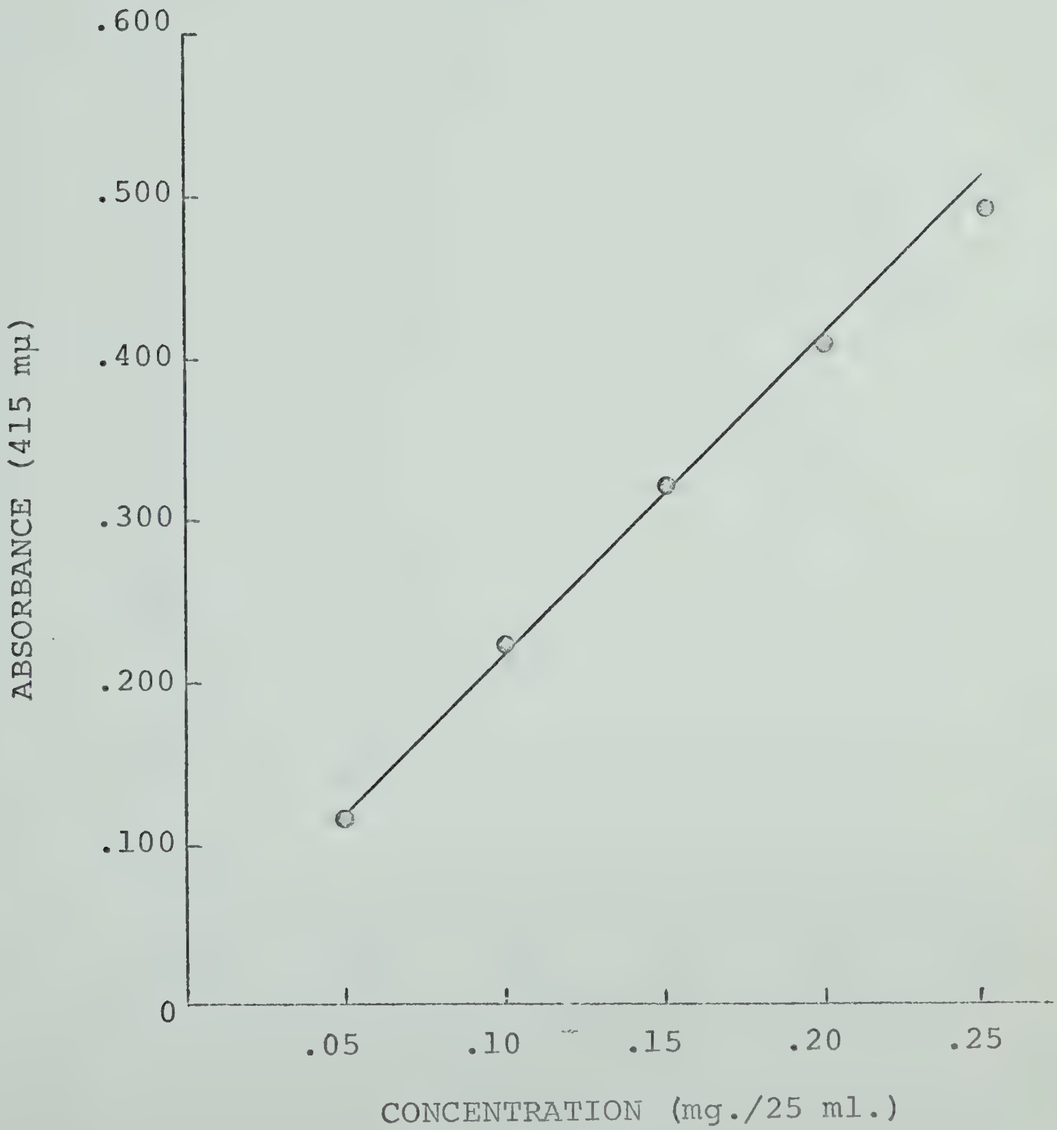


FIGURE 16. CALIBRATION CURVE FOR MEPENZOLATE BROMIDE

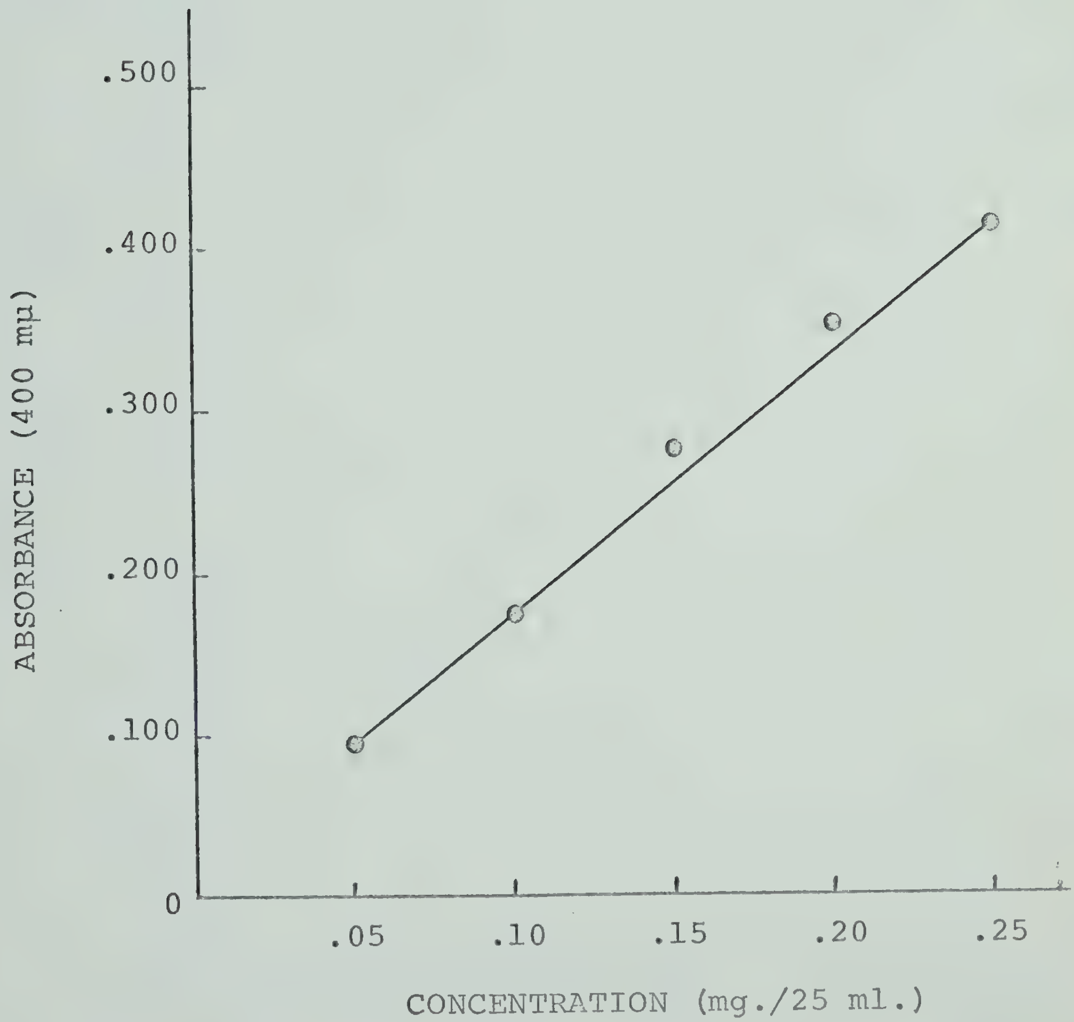


FIGURE 17. CALIBRATION CURVE FOR METHANTHELINE BROMIDE

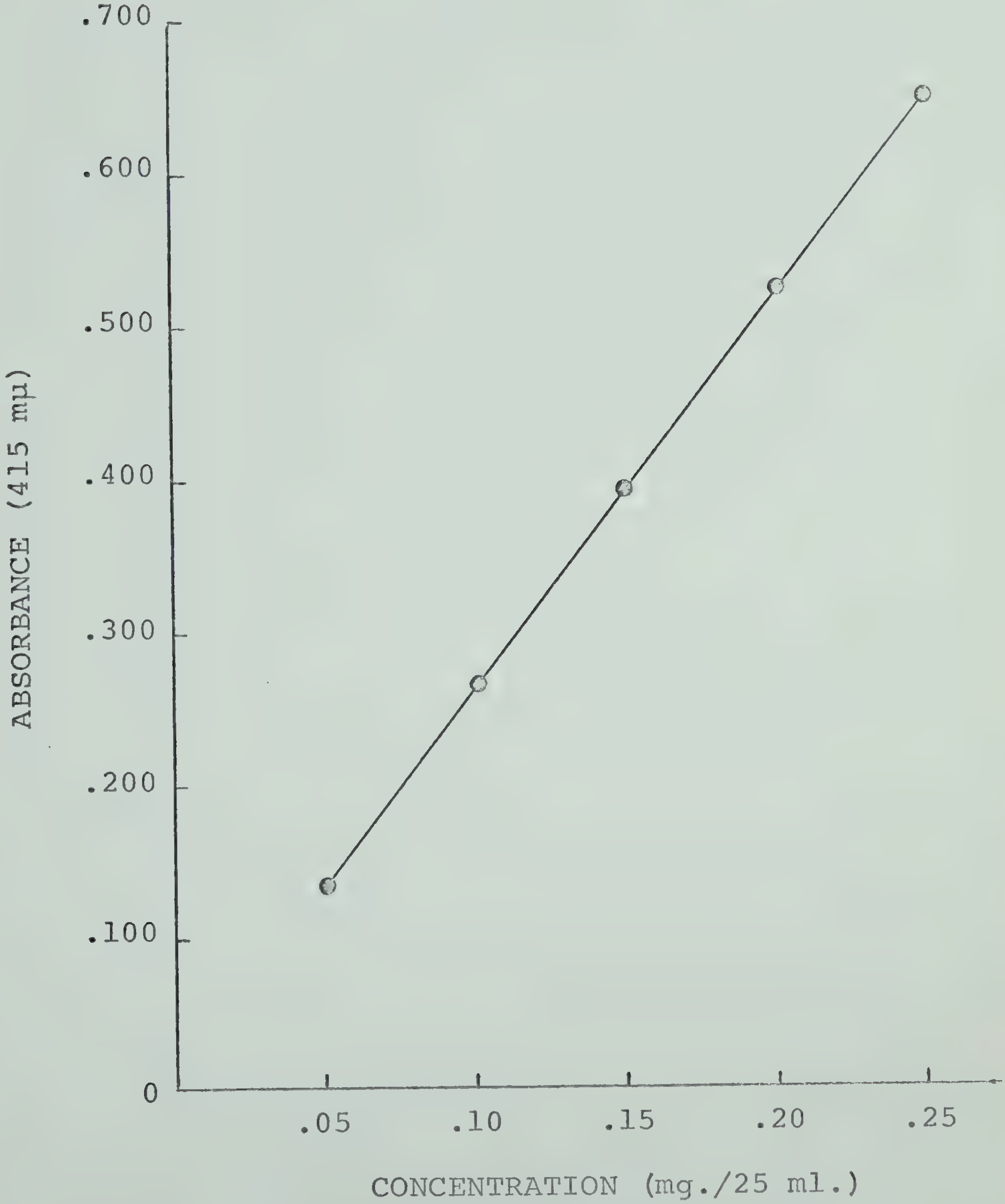


FIGURE 18. CALIBRATION CURVE FOR OXYPHENONIUM BROMIDE

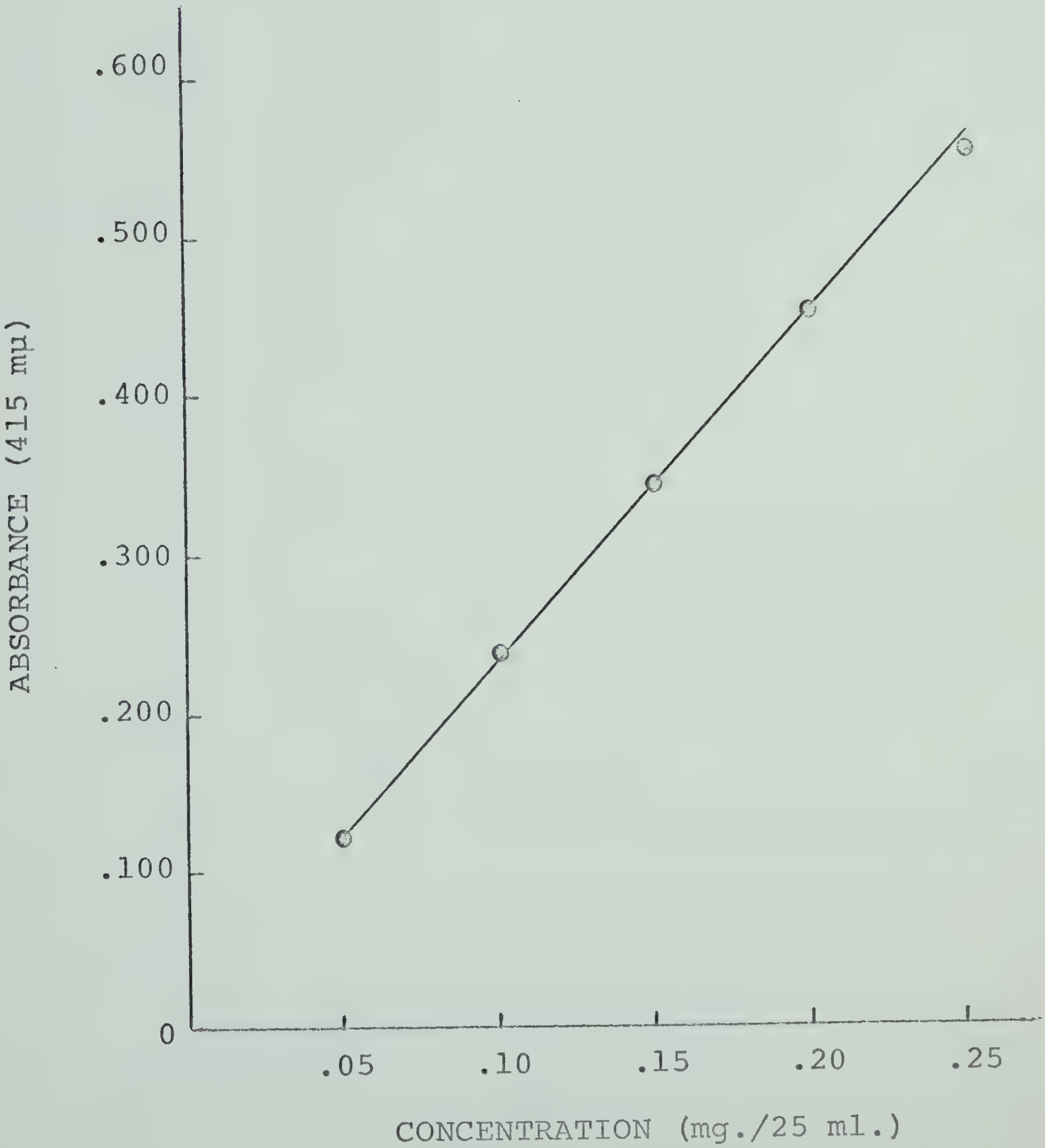


FIGURE 19. CALIBRATION CURVE FOR

PENTHIENATE BROMIDE

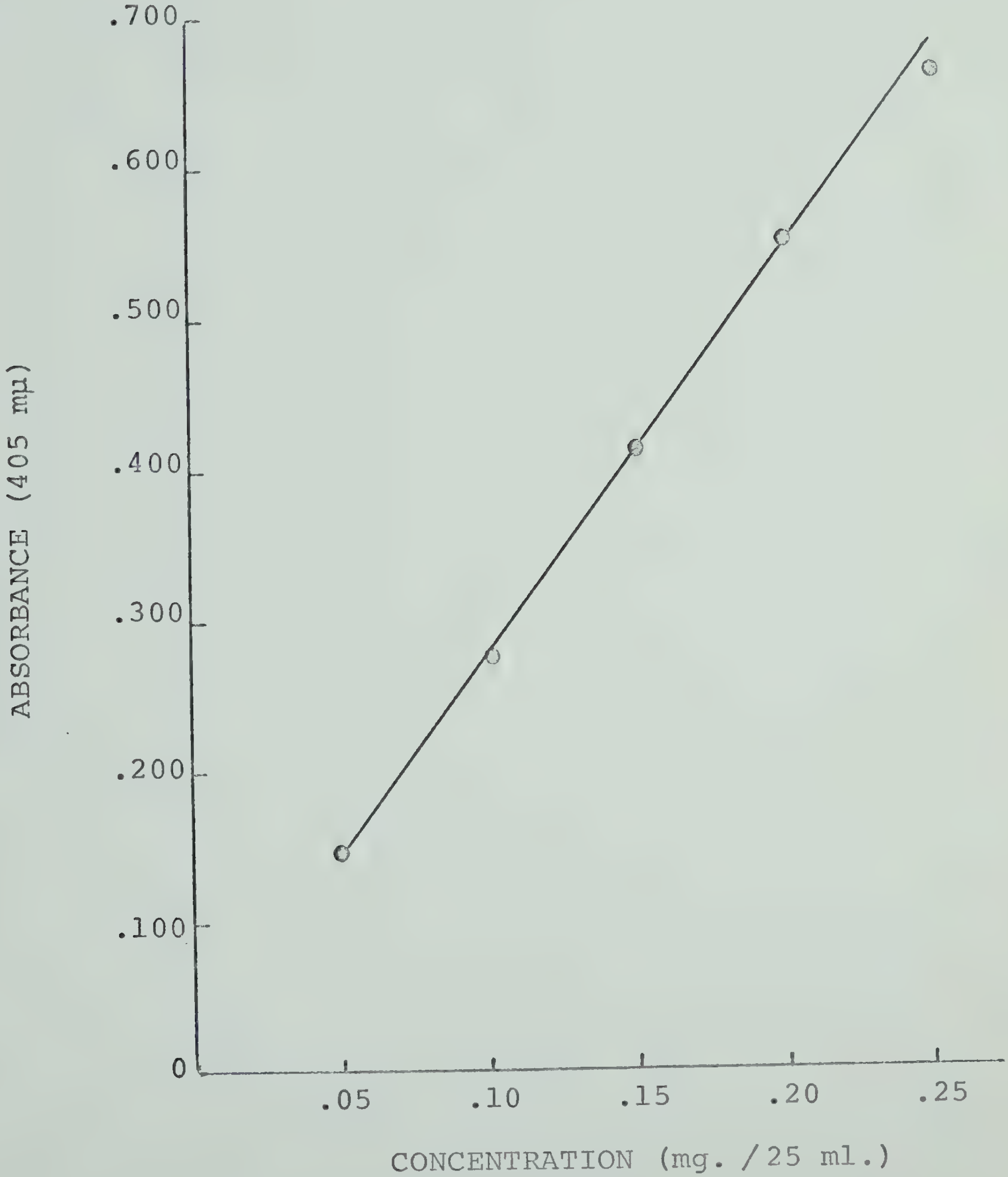


FIGURE 20. CALIBRATION CURVE FOR

PENTOLINIUM TARTRATE

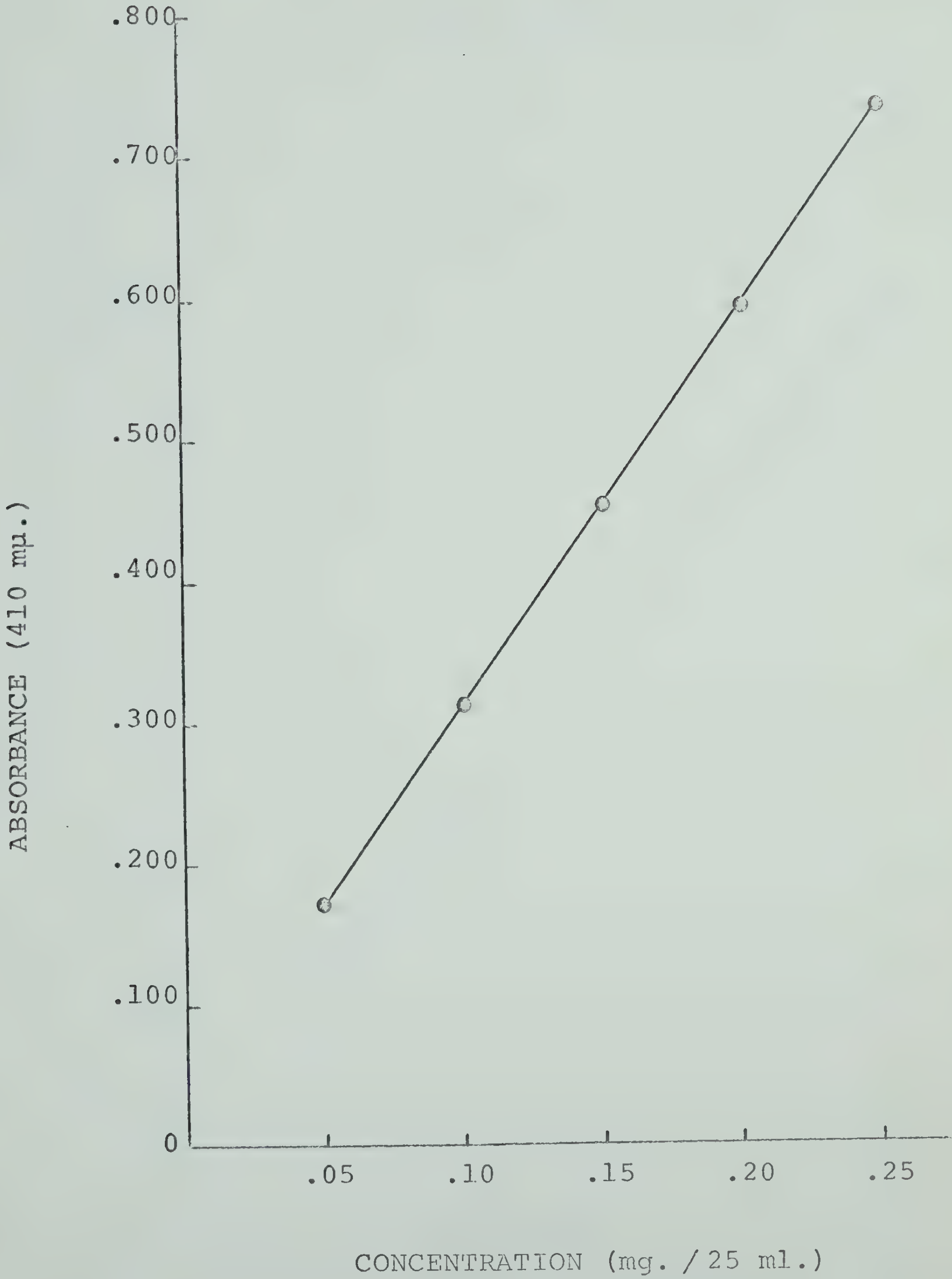


FIGURE 21. CALIBERATION CURVE FOR

PIPENZOLATE BROMIDE

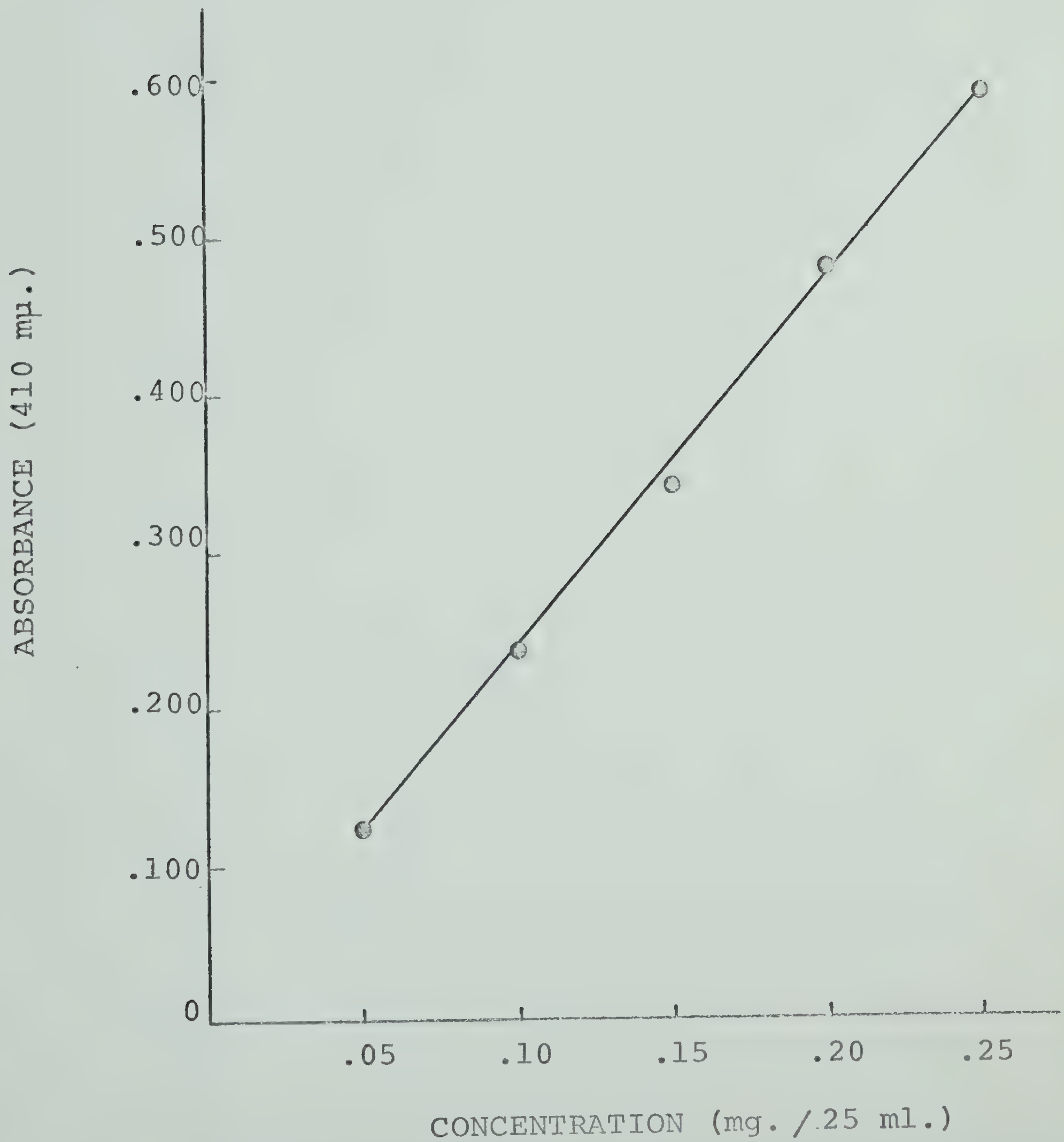


FIGURE 22. CALIBRATION CURVE FOR PROPANTHELINE BROMIDE

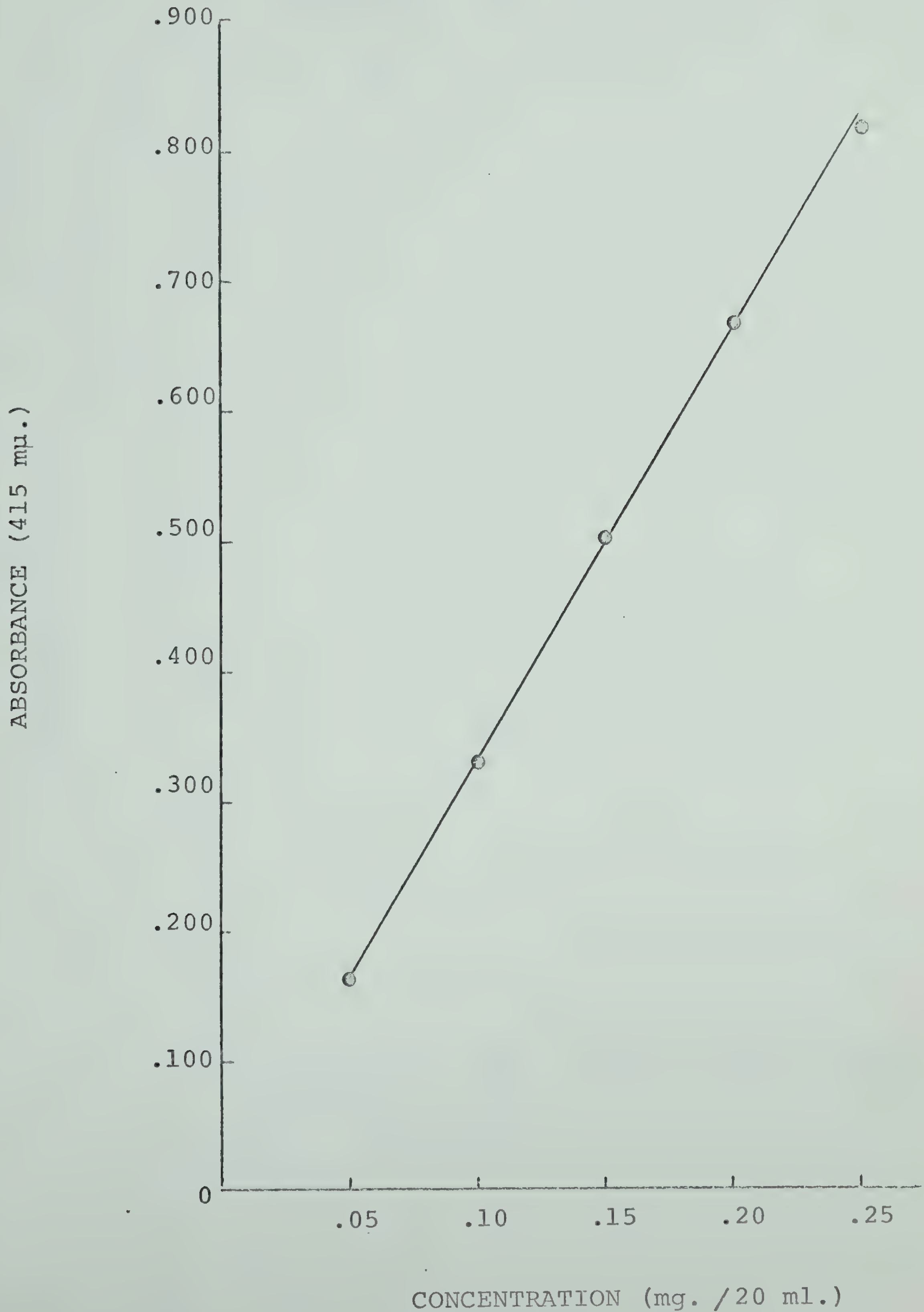


FIGURE 23. CALIBRATION CURVE FOR

PYRIDOSTIGMINE BROMIDE

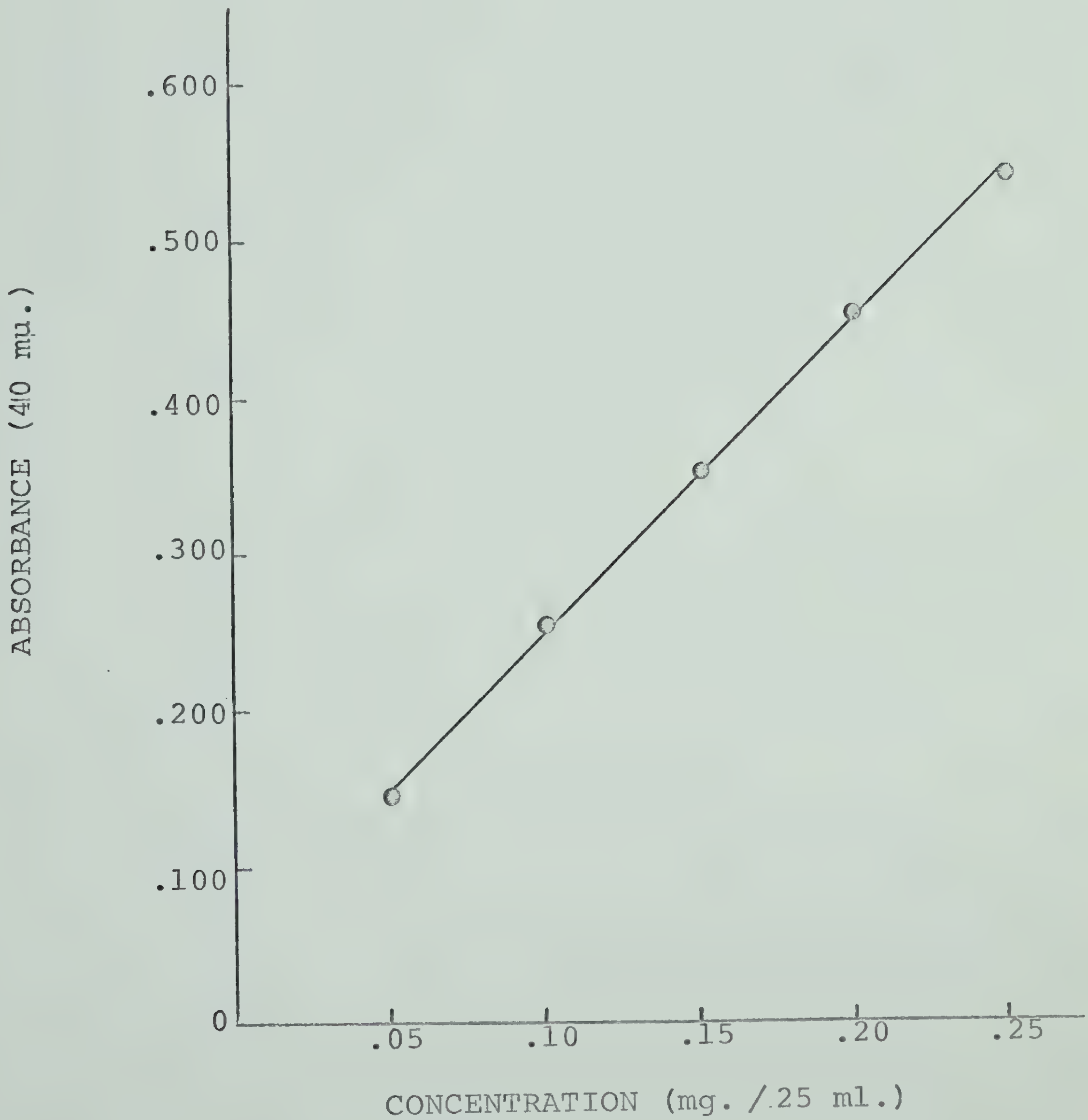
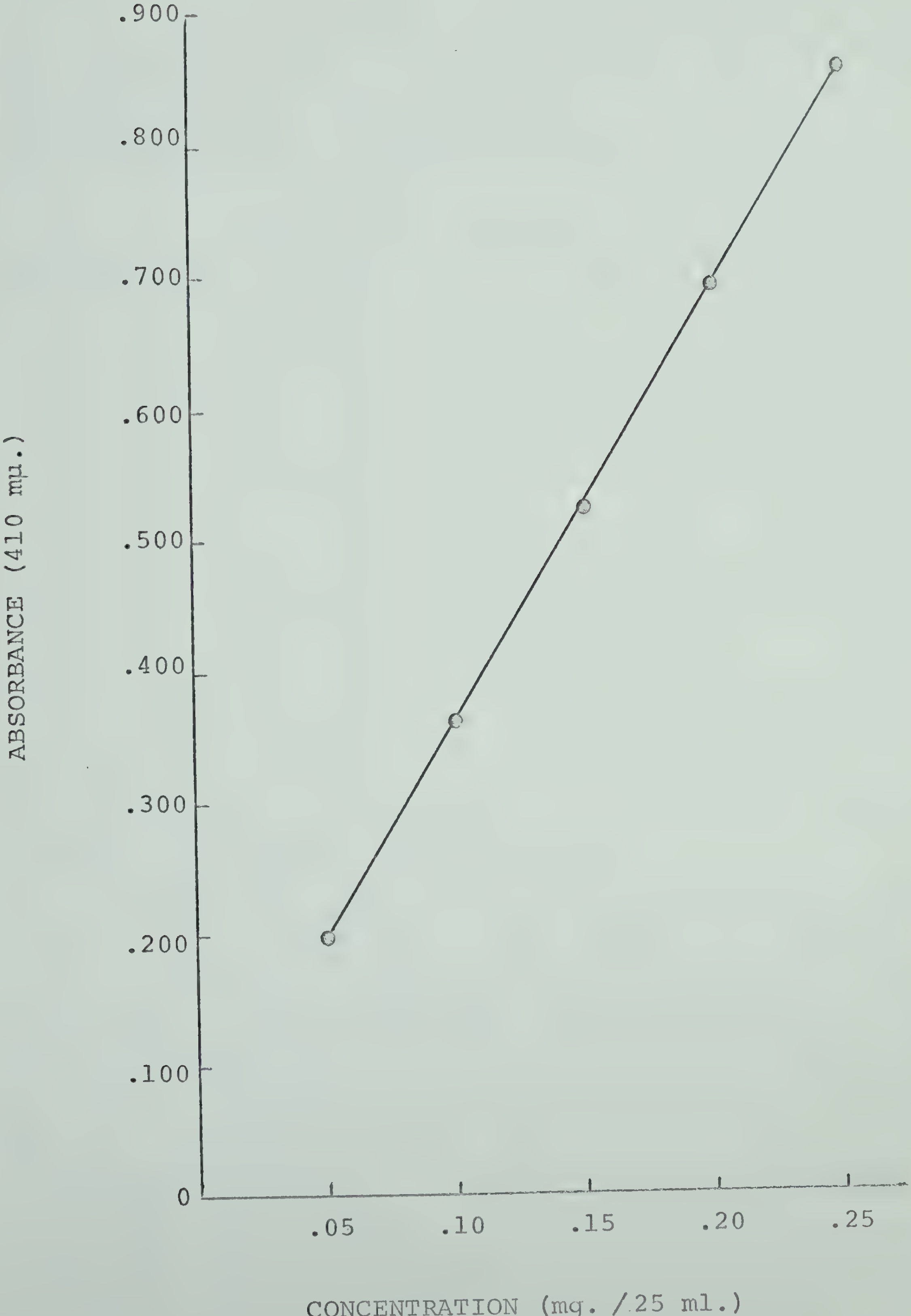


FIGURE 24. CALIBRATION CURVE FOR TRIMETHIDINIUM METHOSULFATE



It was found that 10 minutes was sufficient time for maximum colour development.

For a similar reason, it was also necessary to study the effect of time on the development of colour when penthienate was the drug under investigation.

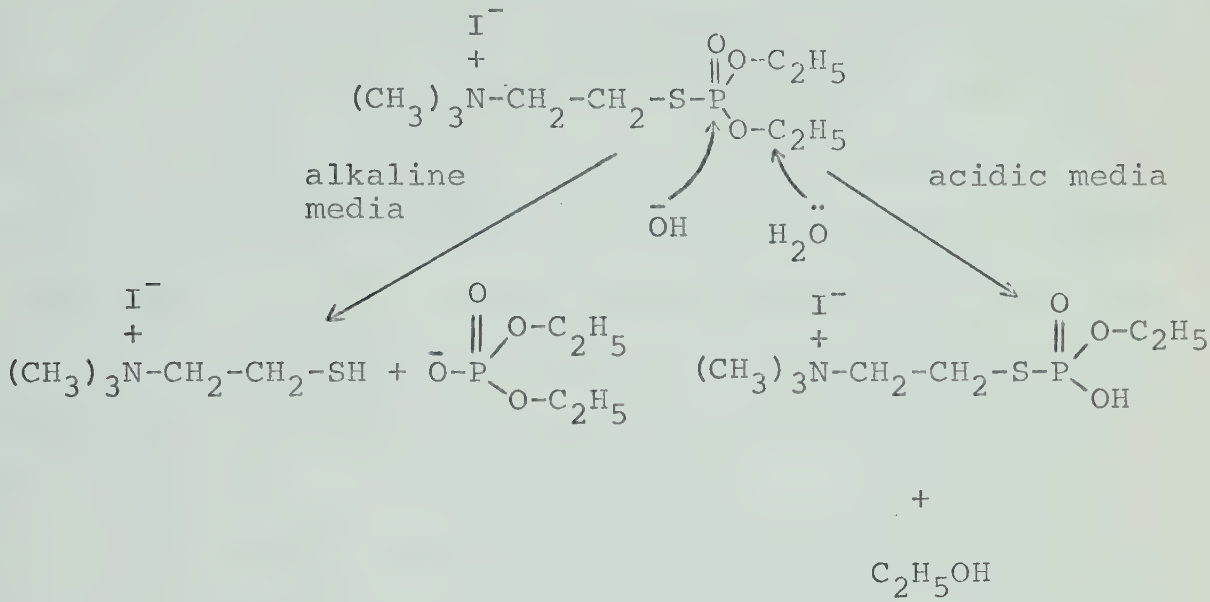
TABLE VIII. STABILITY OF PENTHIENATE-ORANGE IV COMPLEX

Time (min.)	Abs.	Time (min.)	Abs.
1	.345	9	.420
2	.362	10	.420
3	.385	15	.420
4	.391	20	.420
5	.408	25	.420
6	.410	30	.425
7	.418	40	.425
8	.418	50	.430

From the results given in Table VIII, 15 minutes appeared to be ample for stable colour development.

Although the colour produced with echothiophate was linear with concentration, it was difficult to obtain reproducible calibration curves. This phenomenon may be explained by referring to a recent publication by Hussain *et al* (36). In their investigation they found that echothiophate underwent degradation in aqueous solutions. In alkaline media, the major reaction was S-P bond cleavage to yield (2-mercapto-ethyl) trimethylammonium iodide, and in acidic media C-O bond cleavage occurred with the loss of a mole of

alcohol. The mechanisms for these reactions are as follows:



It would appear that complexation would still take place with the degradation products, but the possibility of a mixture of different complexes exists, thus giving rise to different absorbances.

Satisfactory explanations regarding the failure of mepenzolate to produce linear calibration curves are not so readily available, but one factor may be the solubilityof the complex. When the complex was extracted from the aqueous solution a uniform colour was not produced in the methylene chloride layer. Although pipenzolate, which is structurally very similar to mepenzolate, gave absorbance values which were linear with concentration, it was difficult to obtain reproducible calibration curves.

Attempts to establish a linear relationship for bethanecol failed using methyl orange, Orange IV, or bromthymol blue as the complexing agent. In each instance methylene

chloride failed to extract a detectable quantity of the complex. When a quantity of methyl orange and bethanecol were stirred in methylene chloride in the absence of water, a yelow colour characteristic of the complex developed. This would seem to indicate that a complex is formed but either it is extremely insoluble in the organic solvent or that it favours the aqueous phase. Structurally bethanecol is a much smaller molecule and has a lower molecular weight than the other compounds.

Calibration curves for isopropamide and pyridostigmine were also prepared using the method of Santoro (13), which involves re-extraction of the organic phase with hydrochloric acid. Linear calibration curves were obtained in both instances but the procedure was found to be more time consuming and more wasteful of the solvents used.

Pharmaceutical Dosage Forms

Before the pharmaceutical dosage forms were analysed a study on the effect of tablet excipients was carried out. In this investigation a quantity of the tablet excipient was added to 10 ml. of the complexing dye and extracted with methylene chloride. The absorbance of the organic phase gave the following results.

TABLE IX. EFFECT OF TABLET EXCIPIENTS

Excipient	Bromthymol Blue pH 7.0	Orange IV pH 6.0	Methyl Orange pH 8.0
Lactose	.000	.000	.000
Methocel	.010	.003	.003
Mannitol	.002	.000	.000
Sucrose	.004	.002	.004
Phenobarbital	____	____	.004

The results show that the absorbance due to tablet excipients is negligible. Other common ingredients used as tablet excipients are kaolin, starch, magnesium stearate and talc. All of these compounds are, however, water insoluble and would be filtered out in the procedure.

The results for the analysis of the dosage forms are presented in Table X. Where official procedures existed, they were used as a comparative means of analysis. Good results were obtained with the majority of dosage forms and generally good agreement was noted with the comparative method.

TABLE X. ANALYSIS OF PHARMACEUTICAL DOSAGE FORMS

Compound	Average % Recovery	Comparative Methods (% Rec.)
1. Ambenonium chloride		
Mytelase Tablets 10 mg.	102.9 ±0.9	95.20*
Mytelase Tablets 25 mg.	100.2 ±1.3	94.52*
2. Benzethonium chloride		
Phemorol Solution	98.9 ±0.9	101.26**
Phemerol Tincture	98.8 ±0.7	95.00*
3. Cetylpyridinium chloride		
Cepacol Solution	97.2 ±1.0	
Cepacol Lozenge	102.7 ±1.9	
4. Demecarium bromide		
Humorsol Ophthalmic	115.3	
5. Domiphen bromide		
Bradosol Lozenge	97.1 ±0.9	103.33*
Bradosol Powder	100.2 ±1.1	99.81***
6. Echothiophate iodide		
Phospholine 3.0 mg.	121.8	
Phospholine 6.25 mg.	130.0	
Phospholine 12.5 mg.	134.4	
7. Edrophonium bromide		
Tensilon Injection	100.8 ±1.1	99.57**
8. Hexocyclium methosulfate		
Tral with Phenobarbital	102.0 ±1.3	101.45*
9. Methantheline bromide		
Banthine Tablets 50 mg.	102.9 ±0.9	

TABLE X. ...Continued

Compound	Average % Recovery	Comparative Methods (% Rec.)
10. Oxphenonium bromide		
Antrenyl Tablets 5 mg.	100.1 ±1.2	98.80*
Antrenyl Tablets 10 mg.	100.5 ±1.1	100.00*
11. Penthienate bromide		
Monodral Tablets 5 mg.	102.1 ±0.8	102.40**
Monodral Exixir	98.4 ±0.9	108.00*
12. Pentolinium Tartrate		
Ansolysen Tablets 40 mg.	101.4 ±0.7	
Ansolysen Injection	102.8 ±1.4	100.76*
13. Pipenzolate bromide		
Piptal Tablets 5 mg.	95.8 ±1.3	
14. Propantheline bromide		
Pro-Banthine Tablets 7.5 mg.	96.4 ±1.0	
Pro-Banthine Tablets 15 mg.	101.7 ±1.4	
Pro-Banthine Tablets 30 mg.	99.4 ±0.7	
Pro-Banthine Injection	103.7 ±0.1	
15. Pyridostigmine bromide		
Mestinon Tablets 60 mg.	97.8 ±0.4	96.22**
16. Trimethidinium methosulfate		
Ostensin Tablets 20 mg.	86.5 ±2.0	

* Analytical data supplied by manufacturer.

** Analysis by official procedure.

*** Analysis by non-aqueous titration.

It was found that a general procedure could be employed for most of the analyses but in some instances slight modifications were required. For the majority of tablets, extraction of the powdered material was made by distilled water. For Pro-Banthine tablets 7.5 and 15 mg., which are sugar coated, methylene chloride was utilized as the extracting solvent, since extraction with distilled water gave poor recoveries.

In some analyses, blank determinations of the sample without the complexing dye were required because the sample was coloured. Both phemerol Tincture and Monodral Elixir gave no absorbance when extracted with methylene chloride. This indicated that the absorbance would be entirely due to the quaternary ammonium-dye complex in these instances.

Satisfactory results could not be obtained with the dosage forms of demecarium, echothiophate, isopropamide and trimethidinium. The high results obtained from the dosage forms of echothiophate and isopropamide may be attributed to their calibration curves which were not entirely linear. The calibration curve for echothiophate was also difficult to reproduce.

The high recoveries obtained for Humorsol Ophthalmic Solution 0.25% can be explained by the fact that this preparation contains benezalkonium chloride as a preservative (37). Since this method cannot differentiate between quaternary ammonium compounds, the benzalkonium would also complex giving rise to a greater absorbance.

An explanation for the low recoveries obtained with Ostensin Tablets is not apparent because the calibration curves were linear and reproducible.

Theoretical Aspects

Studies on the interaction between the dye and the quaternary ammonium salts have been presented by Zografi, Patel and Weiner (38). They studied the interactions between Orange II and four long chain quaternary ammonium salts - cetylpyridinium chloride, domiphen bromide, dodecylquinolinium bromide and (2-phenoxyethyl) dodecyldimethylammonium bromide. These authors determined the stoichiometry of the interactions by measuring the distribution of the dye between water and chloroform as a function of pH and ionic strength, in the presence of various detergent ion concentrations. Below pH 8.05 all detergents were found to give complexes with a 1:1 stoichiometry. Above pH 12.34, the dye-cetylpyridinium stoichiometry was found to be 1:2 owing to the complete dissociation of the phenolic group of Orange II at this pH. The other dye-detergent complexes continued to show a 1:1 stoichiometry. Their results were substantiated by isolation and elemental analysis of the various dye-detergent complexes.

They concluded that the complexes formed were due to the electrostatic bonding between the negatively charged sulfonate group of Orange II and the cationic nitrogen of the various detergents. They explained the differences in solu-

bility of these complexes as being due to the electronic and steric configuration of the cationic species.

In the present investigation it was found that the complexes formed with certain dyes gave a greater absorbance than those formed from other dyes. The complexity of the molecules involved makes it difficult to offer definite explanations for this phenomenon. There is, however, evidence that the reactions are dependent on the electronic and steric configurations of the species involved. Of the eight compounds for which methyl orange was utilized as the complexing agent, five contained a $O\text{-}CH_2CH_2\text{-}\overset{|}{\underset{|}{N}}^{+}\text{-}$ group. Of the compounds for which either Orange IV or bromthymol blue was employed, only one compound contained a $O\text{-}CH_2CH_2\text{-}\overset{|}{\underset{|}{N}}^{+}\text{-}$ group. Methyl orange did not give complexes with significant absorbances for the bisquaternary compounds and only bromthymol blue was a satisfactory complexing agent for the lower molecular weight compounds.

A study of the molar absorptivities of the quaternary ammonium compounds shown in Table XI, gives more indication of the stoichiometry of the complexes. The compounds complexed with methyl orange all contain one quaternary nitrogen and show approximately the same molar absorptivity. With bromthymol blue and Orange IV, the bisquaternary compounds show molar absorptivities which are approximately twice that of the monoquaternary compounds. This would therefore indicate a dye-quaternary stoichiometry of 2:1 for the bisquaternary compounds.

TABLE XI. MOLAR ABSORPTIVITIES OF QUATERNARY AMMONIUM COMPOUNDS

Compound	Mol. Wt.	Molarity ($x10^{-5}$)	Abs.*	Molar Abs.** ($x10^{4}$)
A. Benzethonium	466.09	1.28	.408	3.19
Cetylpyridinium	357.99	2.09	.593	2.84
Domiphen	414.46	1.45	.470	3.24
Hexocyclium	428.61	1.40	.330	2.36
Isopropamide	480.42	1.25	.321	2.57
Methantheline	420.34	1.43	.394	2.76
Propantheline	448.42	1.67	.504	3.02
Oxyphenonium	428.41	1.40	.346	2.47
B. Echothiophate	383.23	2.09	.264	1.26
Edrophonium	246.15	3.25	.704	2.17
Pentolinium	538.58	1.49	.597	4.01
Pyridostigmine	261.14	3.06	.457	1.49
Trimethidinium	490.67	1.63	.695	4.26
C. Ambenonium	608.50	1.31	.497	3.79
Pipenzolate	434.39	1.84	.483	2.63
Penthienate	420.41	1.90	.557	2.93
Demecarium	716.61	1.12	.541	4.83

A - Compounds complexed with methyl orange

B - Compounds complexed with bromthymol blue

C - Compounds complexed with Orange IV

Abs.* - Absorbance

Abs.** - AbsorPtivity

SUMMARY AND CONCLUSIONS

(1) A study on various acid dyes as complexing agents for quaternary ammonium compounds has been presented. Methyl orange, Orange IV and bromthymol blue were found to be the most satisfactory.

(2) Linear and reproducible calibration curves were obtained for 19 quaternary ammonium compounds. Of the compounds investigated only bethanecol and mepenzolate failed to show a linear relationship.

(3) The effect on the procedure by common tablet excipients has been investigated and found to be of negligible consequence.

(4) A quantitative method of analysis has been developed for 23 pharmaceutical dosage forms containing a quaternary ammonium compound. The procedure is very sensitive and was adaptable to the various types of dosage forms investigated.

(5) On the basis of the present investigation, it is concluded that the proposed method is generally superior to existing methods. It is recognized that the ultraviolet method is equally satisfactory except it is more prone to error by interferences than is the colourimetric technique.

(6) Since the dye-quaternary ammonium complexes have been found to obey Beers Law it would be possible to compare

the unknown sample to a known reference standard solution and thus eliminate the need for a calibration curve.

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APPENDIX

DATA FOR ANALYSIS OF PURE QUATERNARY AMMONIUM

COMPOUNDS BY NON-AQUEOUS TITRATION

Name of Drug	Sample Wt. (mg.)	Vol. of Titrant (ml.)	Normality of Titrant	Mg. Recovered	% Recovered
1. Benzethonium Chloride	100.0	4.15	0.0514	99.4	99.4
	100.0	4.19	0.0514	100.4	100.4
	100.0	4.17	0.0514	99.7	99.7
					99.9*
2. Bethanecol Chloride	38.7	2.31	0.0867	39.4	101.8
	47.0	2.78	0.0867	47.4	100.8
	32.2	1.96	0.0867	33.4	100.7
					101.1*
3. Cetylpyridinium Chloride	50.0	2.70	0.0514	49.7	99.4
	50.0	2.70	0.0514	49.7	99.4
	50.0	2.74	0.0514	50.4	100.8
					99.9*
4. Demecarium Bromide	100.0	3.30	0.0867	102.5	102.5
	100.0	3.28	0.0867	101.9	101.9
	100.0	3.25	0.0867	100.9	100.9
					101.8*
5. Domiphen Bromide	100.0	2.81	0.0851	99.1	99.1
	100.0	2.82	0.0851	99.5	99.5
	100.0	2.83	0.0851	99.8	99.8
					99.5*

DATA FOR ANALYSIS OF PURE QUATERNARY AMMONIUM

COMPOUNDS BY NON-AQUEOUS TITRATION (con't)

Name of Drug	Sample wt. (mg.)	Vol. of Titrant (ml.)	Normality of Titrant	Mg. Recovered	% Recovered
6. Edrophonium Bromide	100.0	9.70	0.0514	100.6	100.6
	100.0	9.70	0.0514	100.6	100.6
	100.0	9.55	0.0514	99.0	99.0
					100.1*
7. Hexocyclium Methosulfate	100.0	2.74	0.0851	99.6	99.6
	100.0	2.73	0.0851	99.4	99.4
	100.0	2.73	0.0851	99.4	99.4
					99.5*
8. Isopropamide Iodide	100.0	2.36	0.0867	98.3	98.3
	100.0	2.37	0.0867	98.7	98.7
	100.0	2.39	0.0867	99.5	99.5
					98.9*
9. Mepenzolate Bromide	100.0	2.80	0.0867	101.9	101.9
	100.0	2.73	0.0867	99.4	99.4
	100.0	2.75	0.0867	100.6	100.6
					100.6*
10. Methantheline Bromide	100.0	4.62	0.0514	99.8	99.8
	100.0	4.65	0.0514	100.4	100.4
	100.0	4.60	0.0514	99.4	99.4
					99.4*
11. Oxyphenonium Bromide	100.0	4.58	0.0514	100.9	100.9
	100.0	4.59	0.0514	101.1	101.2
	100.0	4.55	0.0514	100.2	100.2
					100.7*

DATA FOR ANALYSIS OF PURE QUATERNARY AMMONIUM

COMPOUNDS BY NON-AQUEOUS TITRATION (con't)

Name of Drug	Sample Wt. (mg.)	Vol. of Titrant (ml.)	Normality of Titrant	Mg. Recovered	% Recovered
12. Penthienate Bromide	100.0	4.52	0.0514	97.7	97.7
	100.0	4.59	0.0514	99.2	99.2
	100.0	4.59	0.0514	99.2	99.2
					98.7*
13. Pentolinium Tartrate	100.0	4.20	0.0867	98.0	98.0
	100.0	4.22	0.0867	98.6	98.6
	100.0	4.28	0.0867	99.9	99.9
					98.9*
14. Pipenzolate Bromide	100.0	2.66	0.0867	100.1	100.1
	100.0	2.62	0.0867	98.6	98.6
	102.9	2.72	0.0867	102.4	99.6
					99.4*
15. Propantheline Bromide	100.0	4.17	0.0514	96.0	96.0
	100.0	4.22	0.0514	97.2	97.2
	100.0	4.25	0.0514	97.9	97.9
					97.0*
16. Pyridostigmine Bromide	64.8	2.83	0.0867	63.6	98.1
	55.4	2.48	0.0867	55.7	100.4
	55.7	2.48	0.0867	55.7	100.0
					99.5*
17. Valethamate Bromide	106.2	3.21	0.0867	107.5	101.2
	63.1	1.90	0.0867	63.7	100.9
	70.0	2.10	0.0867	70.4	100.5
					100.9*

* - Average % recovery

DATA FOR SELECTION OF COMPLEXING AGENT

1. Methyl Orange - Benzethonium Complex

a) Determination of Optimum wavelength.

λ(mμ)	pH 2.05	4.00	6.00	7.98	9.95
400	.615	.262	.640	.585	.585
405	.640	.280	.670	.605	.610
410	.670	.300	.675	.625	.625
415	.670	.275	.670	.625	.625
420	.630	.270	.655	.615	.615

b) Stability Determination

Time(min.)	pH 2.05	4.00	6.00	7.98	9.95
0	.630	.335	.635	.640	.560
15	.630	.313	.635	.640	.550
30	.630	.318	.635	.640	.525
60	.630	.320	.635	.640	.510
90	.630	.315	.635	.640	.520
120	.640	.328	.635	.635	.515

2. Orange IV - Benzethonium Complex

a) Determination of Optimum wavelength

λ(mμ.)	pH 2.00	4.00	6.03	8.00	10.00
395	.412	.240	.425	.395	.435
400	.415	.242	.435	.402	.440
405	.415	.242	.430	.402	.440
410	.400	.232	.420	.390	.425

b) Stability Determination

Time(min.)	pH 2.00	4.00	6.03	8.00	10.00
0	.422	.280	.365	.395	.435
15	.422	.285	.365	.395	.435
30	.422	.283	.365	.392	.435
60	.425	.283	.367	.395	.455
90	.425	.283	.368	.395	.475
120	.427	.290	.367	.395	.485

3. Bromthymol Blue - Benzethoniom Complex

a) Determination of Optium wavelength

λ(mμ)	pH 2.15	4.00	6.10	8.50	9.92
400	.122	.192	.207	.540	.640
405	.123	.197	.218	.560	.670
410	.127	.202	.220	.570	.685
415	.126	.193	.222	.580	.690
420	.125	.192	.220	.570	.680
425	____	____	.213	.550	.660

b) Stability Determination

Time (min)	pH 2.15	4.00	6.10	8.50	9.92
0	.187	.328	.222	.570	.690
15	.180	.330	.222	.570	.685
30	.194	.335	.222	.570	.682
60	.192	.335	.222	.572	.690
90	.194	.338	.222	.572	.690
120	.197	.345	.222	.578	.695

4. Orange II - Benzethonium Complex

a) Determination of Optimum wavelength

λ(mμ)	pH 2.10	4.10	5.80	8.00	9.92
465	____	____	.405	.360	____
470	.540	.540	.450	.395	.200
475	.590	.565	.450	.400	.218
480	.620	.600	.455	.407	.221
485	.620	.600	.455	.395	.220
490	.615	.590	.430	.385	.219

b) Stability Determination

Time (min)	pH 2.10	4.10	5.90	8.00	9.92
0	.306	.206	.373	.355	.440
15	.303	.202	.378	.355	.440
30	.300	.195	.378	.355	.435
60	.295	.212	.370	.345	.452
120	.280	.200	.372	.345	.455

5. Bromphenol Blue - Benzethonium Complex

a) Determination of Optimum wavelength

λ(mμ)	pH 2.10	pH 3.95	pH 6.05	λ(mu)	pH 8.05	pH 9.95
395	.850	.640	.355	580	.330	.515
400	.900	.675	.375	585	.390	.610
405	.900	.690	.390	590	.430	.670
410	.920	.685	.385	595	.420	.650
415	.900	.680	.380	600	.370	.565
420	.880	.640	.365			

b) Stability Determination

Time(min)	pH 2.10	3.95	6.05	8.05	9.95
0	.770	.625	.375	.618	.975
30	.770	.620	.390	.530	.850
60	.770	.620	.410	.500	.830
180	.770	.615	.430	.420	.780

DATA FOR CALIBRATION CURVES

1. Ambenonium Chloride

Complexing Agent- 10 ml. 0.0001 N Orange IV pH 6.14
Extracting Solvent- 25 ml. methylene chloride
Optimum Wavelength- 410 mμ.
Equilibration Time- 30 minutes.

Conc. (mg. /25 ml.)	Trial 1	2	3	4	5	Ave.
.05	.122	.132	.118	.127	.120	.123
.10	.253	.252	.255	.257	.250	.253
.15	.380	.385	.380	.403	.390	.388
.20	.515	.485	.505	.510	.470	.497
.25	.635	.630	.618	.625	.650	.632

2. Benzethonium Chloride

Complexing agent- 10 ml. 0.001 N Methyl Orange pH 7.97
Extracting solvent- 25 ml. methylene chloride
Optimum wavelength- 415 mμ.
Equilibration time- 10 minutes

Conc. (mg. /25 ml.)	Trial 1	2	3	4	5	Ave.
.05	.140	.138	.135	.140	.145	.140
.10	.272	.273	.265	.282	.270	.272
.15	.405	.408	.403	.392	.430	.408
.20	.550	.540	.535	.525	.525	.535
.25	.675	.665	.665	.655	.665	.665

3. Cethylpyridinium Chloride
   Complexing agent- 10 ml. 0.0001 N methyl orange pH 7.21
   Extracting solvent- 20 ml. methylene chloride
   Optimum wavelength- 405 mµ.
   Equilibration time- 10 minutes

Conc. (mg. /20 ml.)	Trial 1	2	3	4	5	Ave.
.05	.198	.230	.218	.194	.202	.208
.10	.380	.425	.425	.370	.378	.395
.15	.575	.600	.605	.600	.560	.588
.20	.770	.800	.775	.760	.740	.769
.25	.960	.980	.970	.950	.950	.862

4. Chlorisondamine Chloride
   Complexing agent- 15 ml. .0001 N. Bromthymol Blue
   Extracting solvent- 25 ml. methylene chloride
   Optimum wavelength- 415 mµ.
   Equilibration time- 10 minutes

Conc. (ml. of 0.001 N soln.)	Trial 1	2	3	4	5	Ave.
1	.112	.120	.134	.127	.130	.125
2	.242	.242	.248	.235	.245	.242
3	.360	.370	.360	.355	.358	.361
4	.460	.458	.480	.470	.480	.470
5	.720	.600	.580	.595	.635	.603

5. Demecarium Bromide
   Complexing agent- 10 ml. 0.0001 N Orange IV pH 9.84
   Extracting solvent- 25 ml. methylene chloride
   Optimum wavelength- 415 mµ.
   Equilibration time- 20 minutes
   Stability time- 10 minutes

(mg. /25 ml.)	Trial 1	2	Ave.
.05	.107	.111	.109
.10	.253	.258	.256
.15	.374	.390	.382
.20	.540	.592	.541
.25	.680	.690	.685

6. Domiphen Bromide
   Complexing agent- 10 ml. 0.0001 N methyl orange pH 8.09
   Extracting solvent- 25 ml. methylene chloride
   Optimum wavelength- 415 mμ.
   Equilibration time- 10 minutes

Conc. (mg. /25 ml.)	Trial 1	2	3	4	Ave.
.05	.170	.154	.158	.171	.163
.10	.320	.320	.318	.314	.318
.15	.480	.470	.458	.470	.470
.20	.625	.620	.623	.619	.622
.25	.780	.750	.760	.778	.767

7. Echothiophate Iodine
   Complexing agent- 20 ml. 0.0001 N bromothymol blue pH 7.08
   Extracting solvent- 25 ml. methylene chloride
   Optimum wavelength- 410 mμ.
   Equilibration time- 10 minutes

Conc. (mg. /25 ml.)	Trial 1	2	Ave.
.10	—	.152	.152
.20	.253	.275	.264
.25	.318	—	.318
.30	.363	.388	.376
.40	.480	.526	.503
.50	.550	.618	.584

8. Edrophonium Bromide
   Complexing agent- 20 ml. 0.0001 N bromothymal blue pH 7.08
   Extracting solvent- 25 ml. methylene chloride
   Optimum wavelength- 410 mμ.
   Equilibration time- 10 minutes

Conc. (mg. /25 ml.)	Trial 1	2	3	4	5	Ave.
.05	.210	.216	.220	.230	.215	.218
.10	.370	.385	.380	.400	.385	.382
.15	.515	.555	.530	.575	.560	.547
.20	.675	.718	.680	.760	.690	.704
.25	.800	.870	.840	.910	.870	.858

9. Hexocyclium Methosulfate
Complexing agent- 10 ml. 0.0001 N methyl orange pH 8.08
Extracting solvent- 25 ml. methylene chloride
Optimum wavelength- 420 mμ.
Equilibration time- 10 minutes

Conc.			Trial		
(mg. /25 ml.)	1	2	3	4	Ave.
.05	.136	.104	.104	.096	.110
.10	.233	.213	.230	.211	.222
.125		.272	.295	.256	.274
.150	.333	.323	.325	.340	.330
.175	.422	.360	.380	.390	.376

10. Isopropamide Iodide
Complexing agent- 10 ml. methyl orange pH 8.08
Extracting solvent- 25 ml. methylene chloride
Optimum wavelength- 415 mμ.
Equilibration time- 10 minutes

Conc.		Trial		
(mg. /25 ml.)	1	2	3	Ave.
.05	.116	.116	.114	.115
.10	.217	.228	.225	.223
.15	.326	.310	.327	.321
.20	.405	.405	.422	.411
.25	.495	.490	.502	.496

11. Methantheline Bromide
Complexing agent- 10 ml. 0.0001 N methyl orange pH 8.09
Extracting solvent- 25 ml. methylene chloride
Optimum wavelength- 415 mμ.
Equilibration time- 10 minutes

Conc.			Trial			
(mg. /25 ml.)	1	2	3	4	5	Ave.
.05	.138	.136	.128	.129	.144	.135
.10	.273	.265	.267	.265		.267
.15	.380	.395	.408	.392	.395	.394
.20	.525	.535	.535	.520		.528
.25	.655	.655	.655	.655	.645	.653

12. Oxyphenonium Bromide
Complexing agent- 10 ml. 0.001 N methyl orange pH 7.97
Extracting solvent- 25 ml. methylene chloride
Optimum wavelength- 415 mμ.
Equilibration time- 10 minutes

(mg. /25 ml.)	Trial 1	2	3	4	5	Ave.
.05	.120	.126	.117	.115	.100	.120
.10	.265	____	.225	.235	.223	.237
.15	.355	.335	.335	.380	.325	.346
.20	.488	.445	.435	.480	.430	.456
.25	.575	.555	.540	.575	.550	.559

13. Penthienate Bromide
Complexing agent- 10 ml. 0.0001 N Orange IV pH 6.45
Extracting solvent- 25 ml. methylene chloride
Optimum wavelength- 405 mμ.
Equilibration time- 10 minutes
Stability time- 15 minutes

Conc. (mg. /25 ml.)	Trial 1	2	3	4	5	Ave.
.05	.165	.126	.130	.165	.154	.148
.10	.285	.258	.270	.285	.290	.278
.15	.435	.370	.425	.425	.420	.415
.20	.550	.550	.580	.560	.543	.557
.25	.660	.660	.670	____	.680	.668

14. Pentolinium Tartrate
Complexing agent- 20 ml. .0001 N bromothymol blue pH 7.10
Extracting solvent- 25 ml. methylene chloride
Optimum wavelength- 410 mμ.
Equilibration time- 10 minutes

Conc. (mg. /25 ml.)	Trial 1	2	3	4	5	Ave.
.05	.174	.165	.175	.170	.170	.171
.10	.318	.295	.314	.320	.320	.313
.15	.470	.445	.465	.445	.460	.457
.20	.620	.550	.610	.623	.580	.597
.25	.760	.720	.760	.760	.700	.740

15. Pipenzolate Bromide
Complexing agent- 10 ml. 0.0001 N Orange IV pH 7.95
Extracting solvent- 25 ml. methylene chloride
Optimum wavelength- 410 mμ.
Equilibration time- 10 minutes

Conc. (mg. /25 ml.)	Trial 1	2	3	4	Ave.
.05	.138	.115	.133	.108	.123
.10	.295	.235	.245	.236	.238
.15	.330	.355	.345	.340	.342
.20	.500	.500	.468	.464	.483
.25	.625	.600	.600	.565	.597

16. Propantheline Bromide
Complexing agent- 10 ml. 0.0001 N methyl orange pH 8.10
Extracting solvent- 20 ml. methylene chloride
Optimum wavelength- 415 mμ.
Equilibration time- 10 minutes

Conc. (mg. /20 ml.)	Trial 1	2	3	4	5	Ave.
.05	.150	.170	.168	.152	.168	.162
.10	.310	.338	.333	.332	.342	.331
.15	.475	.520	.515	.495	.515	.504
.20	.630	.700	.665	.680	.680	.671
.25	.780	.860	.820	.830	.820	.822

17. Pyridostigmine Bromide
Complexing agent- 20 ml. bromothymol blue pH 7.05
Extracting solvent- 25 ml. methylene chloride
Optimum wavelength- 410 mμ.
Equilibration time- 10 minutes

Conc. (mg. /25 ml.)	Trial 1	2	3	4	5	Ave.
.05	.145	.147	.145	.146	.146	.146
.10	.245	.255	.260	.262	.254	.255
.15	.358	.360	.360	.348	.352	.356
.20	.442	.460	.470	.450	.461	.457
.25	.540	.530	.540	.555	.565	.546

18. Trimithidinium Methosulfate

Complexing agnet- 20 ml. 0.0001 N bromthymol blue pH 7.1
Extracting solvent- 25 ml. methylene chloride
Optimum wavelength- 410 mμ.
Equilibration time- 10 minutes

Conc.	Trial					
(mg. /25 ml.)	1	2	3	4	5	Ave.
.05	.220	.213	.186	.189	.188	.199
.10	.380	.378	.352	.355	.353	.363
.15	.538	.545	.520	.520	.517	.528
.20	.720	.715	.680	.690	.670	.695
.25	.880	.880	.850	.860	.840	.862

DATA FOR DOSAGE FORMS

1. Ambenonium Chloride

a) Mytelase tablets 10 mg.
Weight of 10 tablets = 1.4828 gm.
Stock solution = 0.0741 gm. of powdered tablet in 100 ml. water
Sample volume = 3 ml. of stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave.	Stand Dev
.390	.1530	102.00	100.55	±0.72
.385	.1530	100.33		
.385	.1530	100.33		
.385	.1530	100.33		
.385	.1530	100.33		
.382	.1500	100.00		

b) Mytelase Tablets 25 mg.
Weight of 10 tablets = 2.5063 gm.
Stock solution = 0.0501 gm. powdered tablet in 100 ml. water.
Sample volume = 3 ml. stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave.	Stand Dev
.398	.1560	104.00	102.20	±1.48
.390	.1530	102.00		
.383	.1500	100.00		
.395	.1550	103.00		
.390	.1530	102.00		

2. BENZETHONIUM CHLORIDE

a) Phemerol Chloride solution 1:1,000
Stock solution = 5 ml. 1:1,000 solution in 100 ml.
Sample volume = 3 ml. stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave.	Stand Dev
.400	.1490	99.33	98.86	±0.86
.398	.1485	99.00		
.392	.1460	97.33		
.400	.1490	99.33		
.400	.1490	99.33		

b) Phemerol Tincture
Stock solution = 10 ml. of tincture q.s. 100 ml. and 25 ml. of this q.s. 100 ml.
Sample volume = 3 ml. of stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave.	Stand Dev
.400	.1490	99.33	98.83	±0.66
.395	.1470	98.00		
.400	.1490	99.33		
.395	.1470	98.00		
.400	.1490	99.33		
.398	.1485	99.00		

3. Cetylpyridinium Chloride

a) Cepacol solution 1:2,000
Stock solution = 10 ml. of 1:2,000 solution q.s. 100 ml.
Sample volume = 3 ml. of stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve	% Recovery	Ave.	Stand Dev
.560	.143	95.33	97.20	±1.04
.570	.1465	97.67		
.570	.1465	97.67		
.570	.1465	97.67		
.570	.1465	97.67		

Blank = .000

b) Cepacol Lozenges
Weight of 5 lozenges = 10.8543 gm.
Stock solution = 3.75 gm. of powdered lozenge in 50 ml. water.
Sample volume = 3 ml. stock solution (theoretically .15 mg.)

Corrected Abs.	Wt. from Cal. Curve(mg)	% Recovery	Ave	Stand Dev
.607	.1560	104.00	102.67	±1.94
.607	.1560	104.00		
.602	.1550	103.33		
.600	.1540	102.67		
.580	.1490	99.33		

Blank abs. = .003

4. DEMECARIUM BROMIDE

a) Humorsol Opthalmic solution 0.25%
Stock solution = 2 ml. of 0.25% solution q.s. 100 ml.
Sample volume = 2 ml. of stock solution (theoretically .100 mg.)

Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave	Stand Dev
.295	.1148	114.80	115.30	±1.04
.294	.1146	114.60		
.300	.1165	116.50		

5. DOMIPHEN BROMIDE

a) Bradosol Lozenges
Weight of 5 lozenges = 18.5192
Stock solution = 7.4076 gm. in 100 ml. water
Sample volume = 5 ml. stock solution

Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave	Stand Dev
.463	.1475	98.33	97.05	±0.85
.452	.1450	96.67		
.455	.1455	97.00		
.452	.1450	96.67		
.450	.1440	96.00		
.460	.1465	97.67		

b) Bradosol Powder
Stock solution = 50 mg. powder in 100 ml. water and 10 ml. of this solution q.s. to 100 ml.
Sample volume = 3 ml. stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave	Stand Dev
.470	.1500	100.00	100.18	±1.06
.465	.1480	98.67		
.480	.1530	102.00		
.470	.1500	100.00		
.471	.1503	100.20		
.471	.1503	100.20		

6. ECHOTHIOPHATE IODIDE

a) Phospholine Eye drops 3 mg.
Stock solution = 0.03 mg. / ml.
Sample volume = 10 ml. stock solution (theoretically .300 mg.)

b) Phospholine Eyedrops 6.25 mg.
Stock solution = 0.0625 mg. / ml.
Sample volume = 4 ml. stock solution (theoretically .250 mg.)

c) Phospholine Eyedrops 12.5 mg.
Stock solution = 0.125 mg. / ml.
Sample volume = 2 ml. stock solution (theoretically .250 mg.)

	Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave.
a)	.459	.379	126.33	121.83
	.430	.352	117.33	
b)	.400	.325	130.00	130.00
	.400	.325	130.00	
c)	.405	.330	132.00	134.40
	.420	.342	136.80	

7. EDROPHONIUM BROMIDE

a) Tensilon Injection
Stock solution = 5 ml. of injection q.s. 100 ml. and 10 ml. of this solution q.s. 100 ml.
Sample volume = 3 ml. of stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve	% Recovery	Ave.	Stand Dev
.545	.1515	101.00	100.78	±1.14
.545	.1515	101.00		
.550	.1530	102.00		
.540	.1530	102.00		
	.1490	99.33		

8. HEXOCYCLIUM METHOSULEATE

a) Trial with Phenobarbital Tablets
Weight of 10 tablets = 1.9608 gm.
Stock solution = 0.3922 gm. of powdered tablet in 100 ml. water
Sample volume = 3 ml. of stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave	Stand Dev
.332	.152	101.33	102.00	±1.34
.332	.152	101.33		
.332	.152	101.33		
.340	.156	104.00		

9. Isopropamide Iodine

a) Darbid Tablets 5 mg.
Weight of 10 tablets = 2.3047 gm.
Stock solution = 0.2305 gm. in 100 ml. water
Sample volume = 4 ml. of stock solution
Absorbances = .540, .520, .520, .540
Absorbances do not fall on calibration curve.

10. METHANTHELINE BROMIDE

a) Banthine Tablets 50 mg.
Weight of 10 tablets = 2.500 gm.
Stock solution = 0.025 gm. in 100 ml. water
Sample volume = 3 ml. of stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave	Stand Dev
.410	.1152	103.53	102.92	±0.87
.410	.1152	103.53		
.410	.1152	103.53		
.405	.1535	102.33		
.402	.1525	101.67		

11. OXYPHENONIUM BROMIDE

a) Antrenyl Tablets 5 mg.
Weight of 10 tablets = 0.9855 gm.
Stock solution = 0.0986 gm. in 100 ml. water
Sample volume = 3 ml. of stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave	Stand Dev
.350	.1520	101.33	101.07	±1.19
.348	.1510	100.66		
.350	.1520	101.33		
.340	.1475	98.66		
.345	.1495	99.67		

b) Antrenyl tablets 10 mg.
Weight of 10 tablets = 1.8644 gm.
Stock solution = 0.0932 gm. of powdered tablet in 100 ml.
Sample volume = 3 ml. of stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave	Stand Dev
.350	.1520	101.33	100.47	±1.11
.342	.1480	98.67		
.353	.1530	102.00		
.345	.1495	99.67		
.348	.1510	100.67		

12. PENTHIENATE BROMIDE

a) Monodral Bromide Tablets 5 mg.
Weight of 10 tablets = 2.2257 gm.
Stock solution = 0.2226 gm. of powdered tablet in 100 ml. water
Sample volume = 3 ml. of stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave	Stand Dev
.425	.1530	102.00	102.06	±0.83
.425	.1530	102.00		
.420	.1515	101.00		
.428	.1550	103.33		
.425	.1530	102.00		

b) Monodral Elixir
Stock solution = 10 ml. elixir q.s. 100 ml.
Sample volume = 3 ml. stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve (mg.)	% Recovery	Ave	Stand Dev
.410	.1480	98.67	98.44	±0.91
.402	.1455	97.00		
.410	.1480	98.67		
.412	.1485	99.00		

13. PENTOLINIUM TARTRATE

a) Ansolysen tablets 40 mg.
Weight of 10 tablets = 0.8120 gm.
Stock solution = 0.1015 gm. in 100 ml. water and 10 ml. of this solution q.s. 100 ml.
Sample volume = 3 ml. stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve(mg.)	% Recovery	Ave	Stand Dev
.455	.1500	100.00	101.39	±0.74
.461	.1520	101.33		
.463	.1525	101.67		
.460	.1520	101.33		
.465	.1530	102.00		
.465	.1530	102.00		

b) Ansolysen Injection
Stock solution = 5 ml. of injection q.s. to 250 ml.
Sample volume = 1.5 ml. stock solution (theoretically .150 mg.)

Abs.	Wt. from Cal. Curve(mg.)	% Recovery	Ave	Stand Dev
.460	.1520	101.33	102.80	±1.35
.470	.1555	103.67		
.475	.1570	104.67		
.467	.1535	102.33		
.465	.1530	102.00		

14. PIPENZOLATE BROMIDE

a) Piptal Tablets 5 mg.
Weight of 10 tablets = 1.1780 gm.
Stock solution = 0.1178 gm. in 100 ml. water
Sample volume = 3 ml. of stock solution (theoretically .125 mg.)

Abs.	Wt. from Cal. Curve(mg.)	% Recovery	Ave	Stand Dev
.290	.121	96.80	95.84	±1.31
.290	.121	96.80		
.290	.121	96.80		
.282	.118	94.40		
.282	.118	94.40		

15. PROPANTHELINE BROMIDE

a) Pro-Banthine Tablets 7.5 mg.
Weight of 10 tablets = 0.7665 gm.
Stock solution = 50 mg. of powdered tablet in 100 ml.
Sample volume = 3 ml. of stock solution (theoretically .1468 mg.)

Abs	Wt. from Cal Curve (mg)	% Recovery	Corrected* Recovery	Ave	Stand Dev.
.458	.1380	94.01	96.92	96.39	±1.03
.458	.1380	94.01	96.92		
.458	.1380	94.01	96.92		
.448	.1350	91.96	94.81		

*Corrected Recovery - correction for purity of reference compound.

b) Pro-Banthine tablets 15 mg.
Weight of 10 tablets = 0.7741 gm.
Stock solution = 25 mg. of powdered tablet in 100 ml. water

Abs	Wt. from Cal. Curve (mg)	% Recovery	Corrected* Recovery	Ave	Stand Dev.
.470	.1415	97.25	100.16	101.65	±1.38
.470	.1415	97.25	100.16		
.480	.1445	99.31	102.29		
.480	.1445	99.31	102.29		
.485	.1460	100.34	103.35		

c) Pro-Banthine tablets 30 mg.
Weight of 10 tablets = 6.2143gm.
Stock solution = 0.1036 gm. of powdered tablet in 100 ml. water
Sample volume = 3 ml. of stock solution (theoretically .150 mg.)

Abs	Wt. from Cal Curve (mg)	% Recovery	Corrected* Recovery	Ave	Stand Dev.
.485	.1460	97.33	100.25	99.36	±0.71
.481	.1450	96.67	99.57		
.477	.1435	95.67	98.54		
.480	.1445	96.33	99.22		

d) Pro-Banthine Injection

i) Stock solution = 28.2 mg. of injection in 100 ml. water. 10 ml. of this solution q.s. 100 ml. Sample volume = 5 ml. of stock solution

ii) Stock solution = 29.4 mg. of injection in 100 ml. water. 10 ml. of this solution q.s. 100 ml. Sample volume = 5 ml. of stock solution

Abs	Wt. from Cal Curve	Theoretical Weight	% Recovery	Corrected* Recovery	Ave	Stand Dev
.470	.142	.141	100.70	103.72	103.71	±0.12
.495	.148	.147	100.68	103.70		
.495	.148	.147	100.68	103.70		

16. PYRIDOSTIOMINE BROMIDE

a) Mestinon Tablets 60 mg.
Weight of 10 tablets = 3.5319 gm.
Stock solution = 0.2930 gm. in 100 ml. water and 10 ml. of this solution q.s. 100 ml.
Sample volume = 3 ml. of stock solution (theoretically .150 mg.)

Abs	Wt. from Cal. Curve(mg.)	% Recovery	Ave	Stand Dev
.340	.1460	97.33	97.80	±0.38
.342	.1465	97.66		
.345	.1475	98.33		
.342	.1465	97.66		
.344	.1470	98.00		

17. TRIMETHIDINIUM METHOSULFATE

a) Ostensin Tablets 20 mg.
Weight of 10 tablets = 1.5906 gm.
Stock solution = 0.0398 gm. of powdered tablet in 100 ml. water
Sample volume = 3 ml. of stock solution (theoretically .150 mg.)

Abs	Wt. from Cal. Curve	% Recovery	Ave.	Stand Dev
.470	.1320	88.00	86.50	±2.04
.472	.1325	88.33		
.450	.1200	84.00		
.459	.1285	85.67		

DATA FOR ANALYSIS OF DOSAGE FORMS BY COMPARATIVE METHODS

1. Phemerol Solution 1:,000

Procedure - N.F XII p. 50
50 ml. of 1:1,000 solution (theoretically 50 mg. of benzethonium) was titrated with 0.02 M. sodium tetraphenylboron.
1 ml. = 0.02 M. Sodium Tetraphenylboron = 8.962 mg.
5.70 ml. = 51.08 mg. = 102.16%
5.55 ml. = 49.74 mg. = 99.48%
5.70 ml. = 51.08 mg. = 102.16%

2. Bradosol Powder

Procedure - non-aqueous titration procedure employed in analysis of pure material.
1 ml. of 0.0851N. = 35.27 mg. of domiphen
2.83 ml. = 99.81 mg. = 99.81%
2.83 ml. = 99.81 mg. = 99.81%
2.83 ml. = 99.81 mg. = 99.81%
Ave = 99.81%

3. Monodral Tablets 5 mg.

Procedure - N.F. XII p. 292
Abs. of standard (20 mcg./ml.) = .373
Abs. of sample = .385 = 20.64 mcg. = 103.20%
= .379 = 20.32 mcg. = 101.61%
= .381 = 20.42 mcg. = 102.41%
Ave = 102.40%

4. Tensilon Injection

Procedure - U.S.P. XVII p.220
Abs. of standard (50 mcg./ml.) = .540
Abs. of sample = .548 = 101.48%
= .545 = 100.93%
= .520 = 96.29%
Ave = 99.57%

5. Mestinon Tablets 60 mg.

Procedure - B.P. 1968 p. 851
Abs. of theoretical 0.0030% solution (E) = .540

$$E^{1\%}_{1\,cm.} = .186$$

$$E^{1\%}_{1\,cm.} = \frac{E}{Cl}$$

$$C = \frac{.540}{.186} = 0.0029\% = 96.67\%$$

Abs. of theoretical 0.0050% solution (E) = .900

$C = \frac{.900}{.186} = 0.0048\% = 96.00\%$

Ave = 96.22%